

Effects of Yougui Pill Combined With Diacerein on Gonitis and Serum Levels of Toll-Like Receptor 4, Matrix Metalloproteinase-3 and Nitric Oxide

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Ma *et al.*: Yougui Pill Effects Combined With Diacerein on Gonitis and Serum Levels

To observe the efficacy of Yougui pill combined with diacerein on gonitis and the effects of serum Toll-like receptor 4, matrix metalloproteinase-3 and nitric oxide. A total of 132 patients with gonitis treated in our hospital from January 2019 to October 2020 were selected and divided according to their treatment regimen. Efficacy: After 8 w of treatment, 28 patients had complete remission in the observation group, accounting for 42.42 %, 25 patients had partial remission, accounting for 37.88 % and 8 patients had remission, accounting for 12.12 %; in the control group, 14 patients had complete remission, accounting for 21.21 %, 21 patients had partial remission, accounting for 31.82 % and 18 patients had remission, accounting for 27.27 %; in the observation group, the overall response rate was higher than that in the control group and it was statistically significant ($p < 0.05$). Serum: After 8 w of treatment, Toll-like receptor 4, matrix metalloproteinase-3 and nitric oxide, erythrocyte sedimentation rate and C-reactive protein in both groups were largely decreased compared with those before treatment ($p < 0.05$) and after 8 w of treatment in the observation group, Toll-like receptor 4, matrix metalloproteinase-3 and nitric oxide, erythrocyte sedimentation and C-reactive protein were lower compared with the control group ($p < 0.05$). Bone and joint function: The pain, daily activities, stiffness and total scores in both groups after 8 w of treatment were greatly decreased compared with those before treatment ($p < 0.05$) and the above scores were lower in the observation group after 8 w of treatment compared with those in the control group ($p < 0.05$). Safety: In the observation group, gastric discomfort occurred in 2 patients, dry mouth in 3 patients and fatigue in 1 patient; in the control group, gastric discomfort occurred in 2 patients and dry mouth in 1 patient; the rate of adverse effects in the observation group was not statistically different from that in the control group ($p > 0.05$). The combination of Yougui pill and diacerein improved the efficacy and decreased the expression of serum related factors without increasing adverse effects in patients with gonitis.

Key words: Yougui pill, diacerein, gonitis, Toll-like receptor 4, matrix metalloproteinase-3, nitric oxide

Gonitis is a common degenerative bone and joint disease in middle-aged and elderly people, which often involves bone, periosteum and periarticular supporting structures and clinically manifests with joint pain, swelling and limited function. Factors such as Toll-Like Receptor 4 (TLR-4), Matrix Metalloproteinase-3 (MMP-3) and Nitric Oxide (NO) play important roles in cartilage damage, periosteal proliferation and chronic inflammation in patients with gonitis^[1]. At present, there is no radical treatment for this disease in western

medicine clinic and most symptomatic treatments such as anti-inflammation and analgesia are used. Diacerein, an interleukin-1 inhibitor with pharmacological effects of inducing chondrogenesis, anti-inflammation, analgesia, antipyresis and *so on*, is one of the common drugs used in the current western medicine for the treatment of osteoarthritis. However, it can cause diarrhea, abdominal pain and other adverse effects, and the symptoms will still worsen after drug withdrawal^[2]. Yougui pill has the efficacy of warming Yang and

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invigorating the kidney and filling the spermatozoon for pulp supplementation, and is commonly used in clinical practice for low back and knee acid cooling caused by deficiency of kidney Yang^[3]. In this study, we applied it to the treatment of gonitis to observe the effect of Yougui pill combined with diacerein on gonitis and the serum levels of TLR-4, MMP-3 and NO, which are reported as follows.

MATERIALS AND METHODS

Inclusion and exclusion criteria:

The inclusion criteria were Gonitis met the criteria of the "Guide for the Diagnosis and Treatment of Osteoarthritis (2018 Edition)"^[4]; Age ≥ 18 y, ≤ 75 y; Syndrome differentiation met the criteria of the "Expert Consensus on Traditional Chinese Medicine Diagnosis and Treatment of Knee Osteoarthritis (2015 Edition)"^[5]; All were primary gonitis and the clinical data were complete.

Exclusion criteria were patients with a history concerning intraarticular treatment or knee surgery; Patients with a history of relevant drug allergies; Patients with other serious somatic diseases; Patients with other bony diseases such as knee fracture, rheumatoid and gout; and patients who were pregnant or lactating women.

General data:

A total of 132 patients with gonitis treated in our hospital from January 2019 to October 2020 were selected and divided according to their treatment regimen and in the control group, 66 patients were given diacerein treatment, including 38 males and 28 females; age range of 36-70 y, mean (56.36 \pm 8.09) y; disease duration of 0.5-15 y, mean (7.96 \pm 2.15) y. In the observation group, 66 patients were given combined therapy with Yougui pill and diacerein, including 34 males and 32 females; age range of 35-70 y, mean (57.12 \pm 8.52) y; disease duration of 0.5-15 y, mean (7.89 \pm 2.27) y. The general data of patients in the two groups were compared and there was no statistical significance ($p > 0.05$).

Methods:

The control group was treated with diacerein capsules (packaged by Kunming Jida Pharmaceutical Co., Ltd., specification: 50 mg, Saudi Food and Drug Authority (SFDA) Approval No. J20150097) orally 50 mg/time for 2 times/d. The observation group was administrated with Yougui pill (Tongrentang Pharmaceutical Factory, Beijing Tongrentang Co., Ltd., specification: 9 g/pill, SFDA Approval No. Z11021040) combined with diacerein treatment and diacerein administration was

the same as that in the control group, which was orally administrated with Yougui pill 1 pill/time, 3 times/d. Both groups were evaluated for efficacy after 8 w of treatment.

Observation indicators and detection methods:

Efficacy criteria: They were formulated with reference to the "Guiding Principles for Clinical Investigation of New Chinese Medicines^[6] and Western Ontario and McMaster Universities Arthritis Index (WOMAC) Scores", Complete remission-After treatment, symptomatic signs such as joint pain, morning stiffness, joint swelling, local skin fever in the joints and unfavorable flexion and extension of the joints disappeared, the WOMAC integral decreased by ≥ 95 % and the joint functional activity returned to normal; Partial remission-With treatment, the above symptom signs significantly relieved, 75 % \leq WOMAC integral reduction < 95 % and the joint functional activity basically returned to normal; Remission-With treatment, the above symptom signs relieved, 30 % \leq WOMAC integral reduction < 75 % and the joint functional activity significantly progressed; Ineffectiveness-Did not meet the above criteria.

Serum factors: Blood was taken before treatment and 8 w after treatment to detect the indicators of TLR-4, MMP-3, NO, Erythrocyte Sedimentation (ESR) and C-Reactive Protein (CRP), venous blood was drawn from the upper limbs over 8 h of fasting, ESR was detected by the Westergren method and serum was collected after centrifugation to detect TLR-4, MMP-3, NO and CRP by enzyme-linked immunosorbent assay. Instrument RT-96A microplate reader, Shenzhen Mairui Medical Electronic Co., Ltd., kit: Shanghai Mlbio Biotechnology Co., Ltd.

WOMAC score^[7]: This includes 3 major aspects of pain, daily activities, stiffness, the total scores were 20, 8 and 68 points, respectively; the higher the score, the more severe the disease.

Safety evaluation: This includes gastric discomfort, dry mouth, fatigue and *so on* during medication.

Statistical methods:

The data were processed using Statistical Package for the Social Sciences (SPSS) 19.0 and the ($\bar{x} \pm s$) was applied to describe the measurement indexes and the t-test was applied for comparison, while the number of cases (percentage) was applied to describe the counting data and the χ^2 test was applied for comparison and statistical significance was considered at $p < 0.05$.

RESULTS AND DISCUSSION

The total effective rate in observation group was higher than that in control group and there was statistical significance ($p < 0.05$). See Table 1.

TLR-4, MMP-3 and NO were greatly decreased in both groups after treatment ($p < 0.05$), and the levels of TLR-4, MMP-3 and NO were lower in the observation group than those in the control group after treatment ($p < 0.05$). See Table 2.

ESR and CRP in both groups decreased greatly after

treatment ($p < 0.05$), and ESR and CRP in the observation group were lower after treatment compared with those in the control group ($p < 0.05$). See Table 3.

The pain, daily activities, stiffness and total scores of the two groups largely decreased after treatment ($p < 0.05$) and the above scores of the observation group were lower than those of the control group after treatment ($p < 0.05$). See Table 4.

The rate of adverse effects in the observation group was not statistically different from that in the control group ($p > 0.05$). See Table 5.

TABLE 1: COMPARISON OF EFFICACY BETWEEN TWO GROUPS [n (%)]

Group	Number of cases	Full mitigation	Partial mitigation	Mitigation	Invalid	Total efficiency
Control group	66	14 (21.21)	21 (31.82)	18 (27.27)	13 (19.70)	53 (80.30)
Observation Group	66	28 (42.42)	25 (37.88)	8 (12.12)	5 (7.58)	61 (92.42)
χ^2						4.117
p						0.043

TABLE 2: COMPARISON OF SERUM TLR-4, MMP-3 AND NO LEVELS BETWEEN THE TWO GROUPS ($\bar{x} \pm s$)

Group	Number of cases	TLR-4 (ng/ml)		MMP-3 (ng/ml)		NO ($\mu\text{mol/ml}$)	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Control group	66	18.96 \pm 5.14	15.26 \pm 3.01*	25.23 \pm 5.14	19.85 \pm 3.23*	58.56 \pm 15.36	50.23 \pm 11.47*
Observation Group	66	19.04 \pm 4.86	11.47 \pm 2.52*	24.98 \pm 4.76	13.05 \pm 2.94*	60.04 \pm 14.27	40.25 \pm 8.14*
t		0.092	7.843	0.290	12.648	0.573	5.765
p		0.927	0.000	0.772	0.000	0.567	0.000

Note: Compared with this group before treatment, * $p < 0.05$

TABLE 3: COMPARISON OF ESR, CRP BETWEEN TWO GROUPS ($\bar{x} \pm s$)

Group	Number of cases	ESR (mm/h)		CRP (mg/l)	
		Before treatment	After treatment	Before treatment	After treatment
Control group	66	18.45 \pm 3.63	12.05 \pm 1.98*	7.05 \pm 1.23	4.25 \pm 0.98*
Observation group	66	18.51 \pm 3.27	9.58 \pm 1.24*	7.14 \pm 1.06	2.89 \pm 0.74*
t		0.100	8.589	0.450	8.997
p		0.921	0.000	0.653	0.000

Note: Compared with this group before treatment, * $p < 0.05$

TABLE 4: COMPARISON OF WOMAC SCORES BETWEEN GROUPS ($\bar{x} \pm s$)

Group	Number of cases	Pain		Daily activities		Stiffness		Total	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Control group	66	15.98 \pm 4.11	9.12 \pm 2.14*	51.36 \pm 13.08	31.52 \pm 10.52*	6.52 \pm 2.02	5.03 \pm 1.54*	72.85 \pm 15.14	46.75 \pm 12.04*
Observation group	66	15.24 \pm 3.97	6.74 \pm 1.45*	50.47 \pm 12.46	20.15 \pm 6.27*	6.65 \pm 1.95	3.58 \pm 1.27*	73.12 \pm 14.52	34.75 \pm 10.15*
t		1.052	7.480	0.400	7.542	0.376	5.901	0.105	6.191
p		0.295	0.000	0.690	0.000	0.707	0.000	0.917	0.000

Note: Compared with this group before treatment, * $p < 0.05$

TABLE 5: TWO SETS OF SAFETY EVALUATIONS [n (%)]

Group	Number of cases	Stomach discomfort	Dry mouth	Fatigue	Total
Control group	66	2 (3.03)	1 (1.52)	0 (0.00)	3 (4.55)
Observation group	66	2 (3.03)	3 (4.55)	1 (1.52)	6 (9.09)
χ^2					1.073
P					0.300

Gonitis is a common clinical degenerative change of the lower extremities that occurs mostly in the middle-aged and elderly, is more common in the 50-60 y age group and is more common in women than men, which is associated with disturbed sex hormone levels and exacerbated bone loss after women enter menopause^[8]. The incidence of gonitis is increasing with the aging socio population in China^[9]. Since cartilage damage, periosteal hyperplasia and chronic inflammation are the main causes of knee pain and dysfunction, the current treatment of gonitis in western medicine is based on symptomatic treatment such as resting, physiotherapy, anti-inflammation, analgesia, nutritious cartilage and lubricating joints, which can relieve the symptoms to a certain extent. However, some patients still have suboptimal efficacy and patients with severe disease need to accept knee replacement therapy^[10].

Diacerein is currently a common drug used in clinical treatment of gonitis and its active ingredient is diacetylrhein, which belongs to the anthraquinone class of compounds and reduces prostaglandin synthesis by inhibiting interleukin-1. Besides anti-inflammatory and analgesic effects, it can also induce chondrogenesis to promote cartilage repair^[11]. Louthrinoo *et al.*^[12] found that diacerein treatment had satisfactory efficacy in rheumatoid arthritis with poor response to methotrexate. Wang *et al.*^[13] used celecoxib combined with diacerein to treat elderly degenerative knee osteoarthritis with satisfactory efficacy, producing complementary effects and the effect was more sustained and safe.

The occurrence of gonitis is closely related to chronic exertion, poor long-term posture, weight bearing, obesity and cold affected Bone Mineral Density (BMD). Yougui pill is an essential medicine for warming the kidney and strengthening Yang in traditional Chinese medicine and belongs to the tonic class, which has the effects of warming the kidney to dissipate cold and strengthening the tendons and bone. Modern research has found its anti-inflammatory, analgesic, osteoporotic and microcirculation improving and pharmacological effects, and it is clinically used to treat hyposexual function of kidney yang deficiency, osteoporosis,

chronic bronchitis, persistent asthma, sciatica, progressive muscular dystrophy, hereditary cerebellar ataxia, breast cysts, leukopenia, lupus erythematosus, chronic diarrhea, chronic gastritis, gastric ulcer, major bleeding, after induced abortion, psoas muscle strain, unexplained postpartum fever, chronic renal failure, nephrotic syndrome and other diseases^[14]. Shao *et al.*^[15] found that the treatment of Yang deficiency cold coagulation type knee osteoarthritis by Yougui pill combined with ginger moxibustion has a significant effect, which can reduce pain and inflammatory symptoms, improve immunity and have a high safety.

In this study, we referred to the "Guiding Principles of Clinical Investigation of New Chinese Medicines" and WOMAC score to evaluate the efficacy and found that the combination of Yougui pill and diacerein improved the efficacy and decreased the pain, daily activity, stiffness scores and WOMAC total scores in patients with gonitis and it had a good safety profile, without increasing the rate of adverse reactions such as stomach discomfort, dry mouth and tongue, and fatigue. This is due to the fact that aconitine type alkaloids contained in aconite in the Yougui pill can exert anti-inflammatory and analgesic effects by exciting the pituitary adrenocortical system^[16]. Staghorn gum extract can promote lymphocyte transformation and upregulate articular chondrocyte kinase expression to repair damaged cartilage and has anti-inflammatory and analgesic effects, and its glycine contained can also promote calcium ion absorption^[17]. Radix Rehmanniae Praeparata extract enhances bone marrow hematopoiesis, modulates immunity, antioxidation and anti-inflammation^[18]. *Angelica sinensis* polysaccharides have immunomodulatory effects and its volatile oil has anti-inflammatory, microcirculation improving and other pharmacological effects^[19].

TLR4 is involved in regulating innate immune response, inflammatory response and arthrohydrops of gonitis patients causes synovial inflammation and joint structure damage through TLR4 pathway^[20]. MMP-3 can act on the extracellular matrix of articular cartilage to degrade proteoglycans and collagens in it

to destroy articular cartilage structure, which cannot activate interstitial collagenases and destroy cartilage structure^[21]. NO functions as both a second messenger and neurotransmitter, and interleukin-1 can induce chondrocytes to synthesize NO and play an important role in the inflammatory reaction^[22]. ESR and CRP are commonly used inflammatory indicators, which can sensitively reflect the body's inflammatory status^[23]. In this study, by detecting serum TLR-4, MMP-3, NO, CRP and ESR, we found that the combination of Yougui pill and diacerein decreased the expression of cartilage damage related factors in serum and alleviated the body's immune inflammatory response. This is one of the important mechanisms for its treatment of gonitis.

In conclusion, the combination of Yougui pill and diacerein improved the efficacy and decreased the expression of serum related factors without increasing adverse effects in patients with gonitis.

Authors' contributions:

Qiwei Ma and Xiaoli Tang have contributed equally to this work.

Conflict of interests:

The authors declared no conflicts of interest.

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