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Original Article

Wutou decoction, in combination with methotrexate, is more effective and equally tolerated, compared to methotrexate alone, in early active rheumatoid arthritis treatment

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Abstract: The aim of this study was to investigate the efficacy and safety of Wutou decoction (WTD) in combination with methotrexate (MTX), compared to MTX alone, in early active rheumatoid arthritis (RA) treatment. A total of 92 RA patients were randomly allocated into the WTD&MTX group and MTX only group, at a 1:1 ratio, in this randomized controlled study. Patients in the WTD&MTX group received WTD 10 g/day and MTX 10-15 mg/week for 12 weeks, while patients in the MTX alone group received MTX 10-15 mg/week for 12 weeks. CRP, ESR, DAS28 scores, low disease activity (LDA) rates, remission rates, health assessment questionnaire-disability index (HAQ-DI) scores, and overall response rates were evaluated at baseline (W0), W4, W8, and W12. Adverse events (AEs) were also recorded. Compared to the MTX alone group, the WTD&MTX group disclosed a decreased DAS28 score ($P < 0.05$) and ESR level ($P < 0.05$) at W12. The WTD&MTX group also presented an increased percentage of patients achieving LDA at W12 ($P < 0.05$). In addition, the WTD&MTX group revealed lower HAQ-DI scores than the MTX alone group at W8 ($P < 0.05$) and W12 ($P < 0.01$). More importantly, the WTD&MTX group exhibited an elevated percentage of patients achieving overall response, assessed by Clinical Guidelines of New Drugs for Traditional Chinese Medicine (TCM), compared to the MTX alone group at W12 ($P < 0.01$). No differences in AEs occurrence between the WTD&MTX group and MTX alone group were observed ($P > 0.05$). In conclusion, WTD in combination with MTX, is more effective and equally tolerated, compared to MTX alone, in treating early active RA patients.

Keywords: Wutou decoction, methotrexate, rheumatoid arthritis, treatment effect, clinical trial

Introduction

Rheumatoid arthritis (RA), one of the most prevalent systemic autoimmune diseases, affects 0.37% of the population in China and 1% of the population worldwide [1-3]. RA is characterized by persistent joint swelling, synovitis, and inflammation. Uncontrolled active RA causes irreversible joint damage and disability, as well as many other extra-articular diseases, such as cardiovascular disease and pulmonary involvement [4, 5]. Currently, there are several kinds of drugs used in clinical practice for RA treatment, consisting of glucocorticoids, non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs), biological agents, and Traditional Chinese Medicine (TCM) [6]. With these treatment drugs, RA patients are able to control symptoms and improve their quality of life. Unfortunately, none of these treatment drugs cure RA. Moreover, RA patients receiving treatment with these drugs suffer from various adverse events (AEs) [7, 8]. Therefore, new treatment drugs with better efficacy and less AEs are necessary.

One of the most classic Traditional Chinese decoctions, Wutou Decoction (WTD) was designed by Chinese Medicine sage Zhongjing Zhang. It has been used to treat arthritis for over a thousand years in China [9, 10]. WTD consists of *Aconiti Radix* (*Aconitum carmichaelii* Debx.), *Paeoniae Radix Alba* (*Paeonia lactiflora* Pall.), *Glycyrrhiza Radix Preparata* (*Glycyrrhiza ura-*

lensis Fisch.), *Ephedrae Herba* (*Ephedra sinica* Stapf.) and *Astragali Radix* (*Astragalus membranaceus Fisch* Bunge) with a mass ratio of 2:3:3:3:3 [11]. Several studies have demonstrated the good anti-RA effects of WTD on animal models of RA [12-14]. TCM physicians have been using WTD as a complementary therapy to current RA treatment in clinical practice for years. However, convincing evidence based on modern clinical trials or observational studies for the favorable efficacy and safety of WTD in RA treatment has not reported until now. This includes comparisons between WTD and conventional DMARDs, such as methotrexate (MTX) for early active RA treatment [12, 13, 15]. Therefore, the purpose of the current study was to investigate the efficacy and safety of oral WTD, in combination with MTX, compared with MTX alone, in early active RA patients.

Methods

Patients

A total of 92 early RA patients, from January 2016 to August 2017, at Shanghai Guanghua Integrative Medicine Hospital, were consecutively recruited in this randomized controlled trial (RCT). Inclusion criteria were as follows: (1) Diagnosed as RA, according to 1987 American College of Rheumatology (ACR) classification; (2) Age above 18 years and less than 75 years; (3) In active disease conditions, defined as a disease activity score in 28 joints (DAS28) above 3.2 points; and (4) Early RA, defined as disease duration of RA less than 2 years. Exclusion criteria were as follows: (1) Treated with DMARDs or biologics within 2 months; (2) Treated with an unstable dosage of NSAIDs or oral glucocorticoids (stable dosage above 4 weeks was allowed to participate); (3) History of other inflammatory joint diseases other than RA; (4) Complicated with moderate to severe renal or hepatic dysfunction; (5) Complicated with human immunodeficiency virus infections, active hepatitis B or C infections, and persistent or severe infection within 3 months before enrollment; (6) History of solid tumors or hematological malignancies; (7) History of tuberculosis; and (8) Pregnant or lactating women.

Ethical approval

This research was approved by the Ethics Committee of Shanghai Guanghua Integrative Medicine Hospital and was conducted according to the Declaration of Helsinki. All participants provided written informed consent.

Data collection

Baseline demographic and clinical data were collected, including age, gender, body mass index (BMI), disease duration, tender joint count (TJC), swollen joint count (SJC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), disease activity score in 28 joints (DAS28), health assessment questionnaire-disability index (HAQ-DI), rheumatoid factor (RF) status, and anti-citrullinated protein antibody (ACPA) status.

Randomization

The blocked randomization method was used in this study. The randomization code was generated by SAS 9.2 software (Statistical Analysis System, USA). Randomization was performed by an independent statistician not involved in the study elsewhere. Documents were sent and kept by a medical and statistical service company (Shanghai Qeejen Bio-tech Company, China). When a patient was eligible for the study, a call was made to the Qeejen Company. A unique subject identification number was provided from the randomized module.

Treatment

After randomization, the patients were randomly assigned to the WTD&MTX group or MTX alone group at a 1:1 ratio. In the WTD&MTX group, patients received oral WTD 10 g/d and oral MTX once a week (dose escalated from 10 to 15 mg per week within 4 weeks) for 12 weeks. In the MTX alone group, patients received oral MTX once a week (dose escalated from 10 to 15 mg per week within 4 weeks) for 12 weeks. For this study, WTD granules were purchased from Jiangyin Tianjiang Pharmaceutical Co., Ltd (China) and MTX was purchased from Shanghai Xinyi Pharmaceutical Co., Ltd (China). WTD granules were mainly composed of radix aconiti preparata, ephedra, astragalus membranaceus, radix paeoniae alba, liquorice, and honey.

Assessment

CRP levels, ESR levels, DAS28 scores, percentage of low disease activity (LDA), percentage of disease remission, HAQ-DI scores, and overall response rates (Chinese Medicine assessment) were evaluated at baseline, W4, W8, and W12. Low disease activity was defined as DAS28 scores less than 3.2 and disease remis-

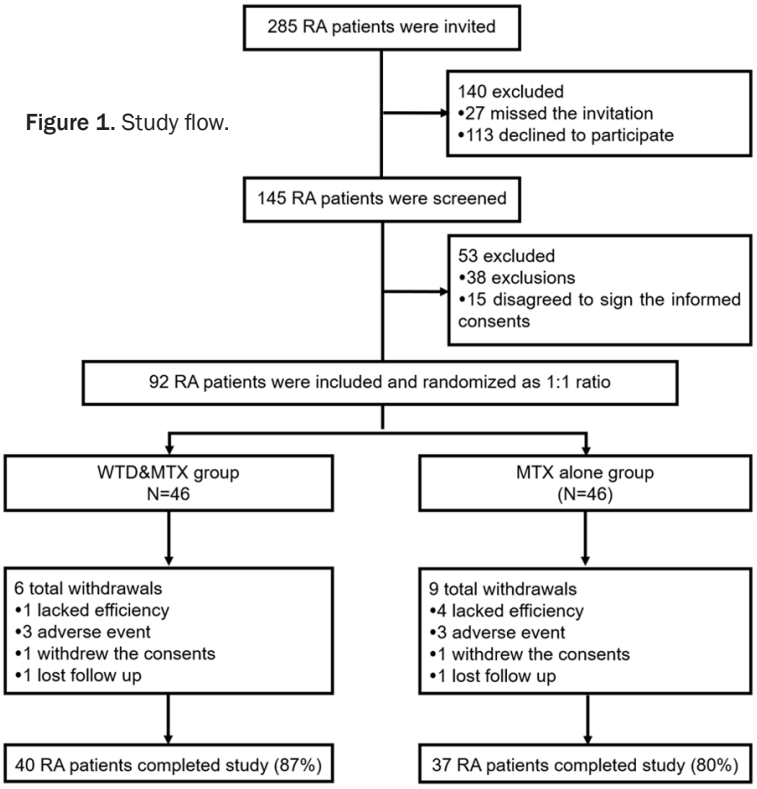

Figure 1. Study flow.

Table 1. Characteristics of RA patients in the WTD&MTX group and MTX alone group

Parameters	MTX alone group (N = 46)	WTD&MTX group (N = 46)	*P* value
Age (years)	47.5 ± 11.4	46.7 ± 10.9	0.732
Gender-Female (n/%)	37 (80.4)	36 (78.3)	0.797
BMI (kg/m^2)	22.0 ± 3.2	22.4 ± 3.1	0.544
Disease duration (years)	0.6 (0.1-1.9)	0.7 (0.1-1.9)	0.581
TJC (joints)	5 (2-12)	5 (1-13)	0.317
SJC (joints)	5 (1-15)	4 (1-16)	0.740
ESR (mm/h)	31.4 ± 14.5	30.6 ± 15.2	0.797
CRP (mg/l)	25.9 ± 13.8	26.3 ± 14.3	0.892
DAS28 score (ESR)	5.36 ± 1.16	5.48 ± 1.03	0.601
HAQ-DI score	1.90 ± 0.71	1.93 ± 0.67	0.834
RF positive (n/%)	33 (71.7)	34 (73.9)	0.815
ACPA positive (n/%)	34 (73.9)	37 (80.4)	0.456

Data are presented as mean value ± standard deviation, medians and (range), or count (percentage). Comparisons were determined by t-test, Wilcoxon rank sum test, or Chi-Squared test. P < 0.05 indicates statistical significance. RA, rheumatoid arthritis; WTD, Wutou Decoction; MTX, methotrexate; BMI, body mass index; TJC, tender joint count; SJC, swollen joint count; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; DAS28, disease activity score in 28 joints; HAQ-DI, health assessment questionnaire-disability index; RF, rheumatoid factor; ACPA, anti-citrullinated protein antibody.

sion was defined as DAS28 scores less than 2.6. Overall response rate was assessed according to Clinical Guidelines of New Drugs for TCM [16]. In addition, adverse events (AEs) were recorded.

Statistics

Statistical analyses were performed based on intention-to-treat (ITT) principles with the last observation carried forward (LOCF) method from any of the three post-baseline measures. Statistical analysis was performed using SAS 9.2 software (Statistical Analysis System, USA) and GraphPad Prim 6.0 (GraphPad, USA). Data are presented as mean value ± standard deviation, median (range), or count (percentage). Comparisons between two groups were determined by t-test, Wilcoxon rank sum test, or Chi-Squared test. P < 0.05 indicates statistical significance.

Results

Study flow

In the current study, 285 RA patients were invited (**Figure 1**). A total of 140 patients were excluded. Twenty-seven patients missed the invitation, while the other 113 patients declined to participate. The remaining 145 patients were screened for eligibility, during which 38 patients were excluded and 15 patients did not agree to provide informed consent. Finally, 92 patients were included in the study and randomized at a 1:1 ratio into the WTD&MTX group and MTX alone group. A total of 6 patients withdrew in the WTD&MTX group, including 1 patient that lacked efficiency, 3 patients that had AEs, 1 patient that withdrew consent, and 1 patient that was lost to follow up. In the MTX alone

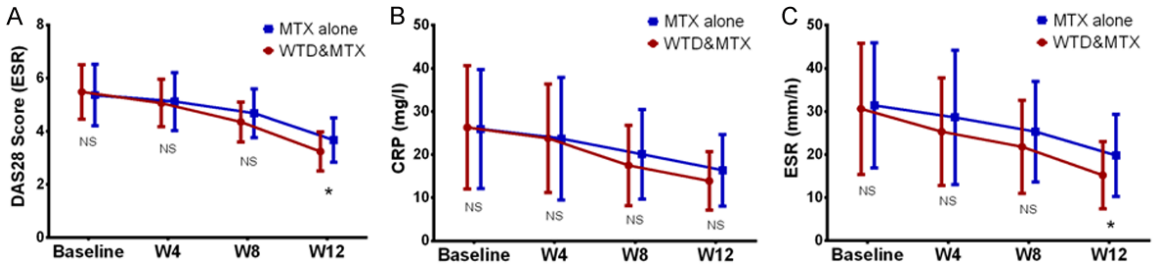

Figure 2. DAS28 scores, CRP levels, and ESR levels in the MTX alone group and WTD&MTX group. Compared to the MTX alone group, the WTD&MTX group presented reduced DAS28 scores (A), similar CRP levels (B), and decreased ESR levels (C) at W12. Comparisons between two groups were determined by t-test. P < 0.05 indicates statistical significance. WTD, Wutou Decoction; MTX, methotrexate; DAS28, disease activity score in 28 joints; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

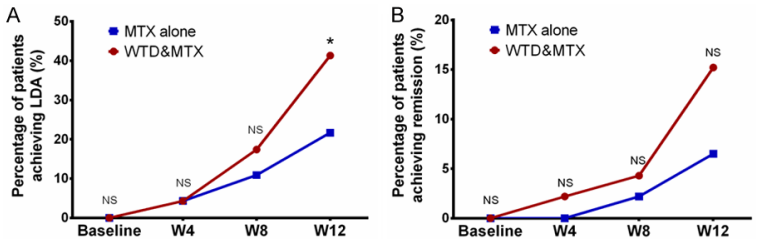

Figure 3. LDA rates and remission rates in the MTX alone group and WTD&MTX group. Compared to the MTX alone group, LDA rates in the WTD&MTX group were increased at W12 (A); Whereas the percentage of patients achieving remission was not different between the two groups (B). Comparisons between two groups were evaluated via Chi-Squared test. P < 0.05 indicates statistical significance. WTD, Wutou Decoction; MTX, methotrexate; LDA, low disease activity.

group, 9 patients withdrew. Of these, 4 patients lacked efficiency, 3 patients had AEs, 1 patient withdrew consent, and 1 patient was lost to follow up. Ultimately, 40 (87%) patients in the WTD&MTX group and 37 (80%) patients in the MTX alone group completed the study (**Figure 1**).

Baseline characteristics

There were no differences between the MTX alone group (n = 46) and WTD&MTX group (n = 46) in demographic and clinical characteristics (all P > 0.05). The mean values of age were 47.5 ± 11.4 years in the MTX alone group and 46.7 ± 10.9 years in the WTD&MTX group (P = 0.732). There were 37 (80.4%) females and 36 (78.3%) females in the MTX alone group and WTD&MTX group, respectively (P = 0.797). Mean BMI was 22.0 ± 3.2 kg/m^2 in the MTX alone group and 22.4 ± 3.1 kg/m^2 in the WTD&MTX group (P = 0.544). Median values of disease duration (P = 0.581), TJC (P = 0.317), and SJC (P = 0.740) were 0.6 (0.1-1.9) years, 5 (2-12) joints, and 5 (1-15) joints in the MTX alone group and were 0.7 (0.1-1.9) years, 5 (1-13) joints, and 4 (1-16) joints in the WTD&MTX group, respectively. Other detailed clinical characteristics are depicted in **Table 1**.

Comparison of DAS28, CRP, and ESR between the MTX alone group and WTD&MTX group.

There were no differences in DAS28 scores (P > 0.05, **Figure 2A**), CRP (P > 0.05, **Figure 2B**), or ESR (P > 0.05, **Figure 2C**) at baseline, W4, or W8 between the MTX alone group and WTD&MTX group. Most importantly, DAS28 scores (P < 0.05, **Figure 2A**) and ESR levels (P < 0.05, **Figure 2C**) were both decreased in the WTD&MTX group, compared to MTX alone group at W12. CRP levels (P > 0.05, **Figure 2B**) showed a decreasing trend in the WTD&MTX group, compared to the MTX alone group at W12, but results were not statistically significant.

Comparison of LDA rates and remission rates between the MTX alone group and WTD&MTX group

As shown in **Figure 3**, no differences in percentage of patients achieving LDA (all P > 0.05, **Figure 3A**) or remission (all P > 0.05, **Figure 3B**) between the MTX alone group and WTD&MTX group at baseline, W4, or W8 were observed. The percentage of patients achieving LDA (P < 0.05, **Figure 3A**) was increased in the WTD&MTX group, compared to MTX alone group, at W12. The percentage of patients achieving remission was numerically elevated in

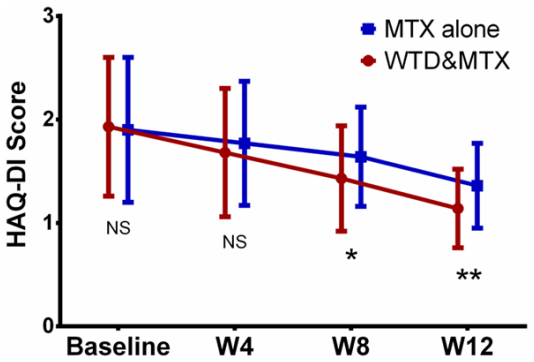

Figure 4. HAQ-DI in the MTX alone group and WTD&MTX group. Compared to the MTX alone group, the WTD&MTX group had a lower HAQ-DI score at W8 and W12. Comparisons between the two groups were assessed by t-test. $P < 0.05$ indicates statistical significance. WTD, Wutou Decoction; MTX, methotrexate; HAQ-DI, health assessment questionnaire-disability index.

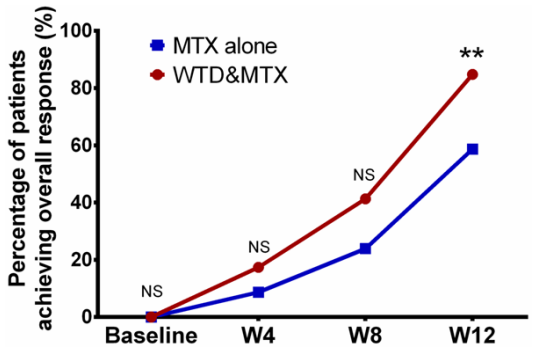

Figure 5. Overall response rates, assessed by Chinese Traditional Medicine assessment guidelines, in the MTX alone group and WTD&MTX group. Compared to the MTX alone group, overall response rates in the WTD&MTX group were elevated at W12. Chi-Squared test was used for comparisons between the two groups. $P < 0.05$ indicates statistical significance. WTD, Wutou Decoction; MTX, methotrexate.

the WTD&MTX group at W12 ($P > 0.05$, **Figure 3B**), but results were not statistically significant.

Comparison of HAQ-DI scores between the MTX alone group and WTD&MTX group

HAQ-DI scores at baseline or W4 ($P > 0.05$, **Figure 4**) exhibited no differences between the MTX alone group and WTD&MTX group, while it was decreased in the WTD&MTX group, compared to the MTX alone group, at W8 ($P < 0.05$, **Figure 4**) and W12 ($P < 0.01$, **Figure 4**).

Comparison of overall response rates between the MTX alone group and WTD&MTX group

No differences were discovered in overall response rates, assessed by Clinical Guidelines of New Drugs for TCM, at baseline ($P > 0.05$, **Figure 5**), W4 ($P > 0.05$, **Figure 5**), or W8 ($P > 0.05$, **Figure 5**) between the MTX alone group and WTD&MTX group. However, overall response rates were elevated in the WTD&MTX group ($P < 0.01$, **Figure 5**), compared to the MTX alone group, at W12.

Comparison of AEs between the MTX alone group and WTD&MTX group

Incidence of AEs between the MTX alone group and WTD&MTX group was not statistically significant. As presented in **Table 2**, there were 3 (6.5%) patients in the MTX alone group and 8 (17.4%) patients in the WTD&MTX group that had abnormal liver function tests ($P = 0.108$). The number of patients with nausea and vomiting was 3 (6.5%) in the MTX alone group and 1 (2.2%) in the WTD&MTX group ($P = 0.307$). In addition, 4 (8.7%) patients in the MTX alone group and 1 (2.2%) patient in the WTD&MTX group had infections ($P = 0.168$). Other AEs are shown in **Table 2**.

Discussion

The current study discovered that DAS28 scores and ESR levels in the WTD&MTX group were decreased and LDA rates were increased, compared to the MTX alone group, at W12. Compared to the MTX alone group, the WTD &MTX group had lower HAQ-DI scores at W8 and W12 and a higher overall response rate at W12. AEs in the WTD&MTX group were in accord with those in the MTX alone group.

According to TCM theory, WTD is used to balance cold-hot and yin-yang, especially for Bi syndrome, such as RA. In modern medical studies, WTD has been found to alleviate inflammation and bone destruction in various animal models of RA [12-14, 17]. WTD may reduce immature blood vessels in synovial membrane tissues of inflamed joints, thereby decreasing inflammation of joints in collagen-induced arthritis (CIA) rats [17]. Similar results have been observed in adjuvant-induced arthritis (AIA) rats. Pathological characteristics of RA, such as inflammatory cell infiltration, synovium proliferation, cartilage destruction, synovial pan-

Table 2. AEs of RA patients in the WTD&MTX group and MTX alone group

Parameters	MTX alone group (N = 46)	WTD&MTX group (N = 46)	*P* value
Abnormal liver function,	3 (6.5)	8 (17.4)	0.108
Nausea and vomiting	3 (6.5)	1 (2.2)	0.307
Infection	4 (8.7)	1 (2.2)	0.168
Decreased WBC count	1 (2.2)	0 (0.0)	0.315
Decreased platelet count	0 (0.0)	1 (2.2)	0.315
Decreased hemoglobin level	1 (2.2)	0 (0.0)	0.315
Rash	2 (4.3)	0 (0.0)	0.153
Fever	1 (2.2)	0 (0.0)	0.315
Abnormal renal function	0 (0.0)	3 (6.5)	0.078
Total	13 (28.3)	11 (23.9)	0.635

Data are presented as count (percentage). A patient may occur more than 1 AE. Comparisons were determined by Chi-squared test. $P < 0.05$ indicates statistical significance. AEs, adverse events; RA, rheumatoid arthritis; WTD, Wutou Decoction; MTX, methotrexate.

nus expansion, and bone corrosion, are suppressed by administrating WTD for three weeks [13]. More interestingly, WTD-treated CIA rats have decreased mean arthritis scores, percentage of arthritis limbs, inflammation scores, and bone destruction scores, in a dose-dependent manner, compared to non-treated CIA rats. Notably, when compared with MTX-treated rats, WTD-treated rats exhibit lower inflammation scores and bone destruction scores [12]. Although these animal studies have demonstrated the ability of WTD to reduce inflammation levels and alleviate joint damage in arthritis rats and the efficacy of WTD is superior to MTX to some extent, there is no evidence based on RCT studies that illuminates the efficacy of WTD in RA treatment until now. Therefore, the present RCT study was conducted, indicating that WTD&MTX group patients had decreased DAS28 scores and ESR levels, as well as increased LDA rates at W12, compared to the MTX alone group. The WTD&MTX group also exhibited lowered HAQ-DI scores and an elevated overall response rate, compared to the MTX alone group, suggesting oral WTD in combination with MTX was more effective in reducing the disease activity of RA and in improving the quality of life than oral MTX alone. Present results might benefit from the anti-inflammation effects of WTD in RA patients as follows. WTD is capable of downregulating inflammatory cytokines in RA patients [18]. It has been reported that pretreatment with WTD leads to significant inhibition of macrophage inflammatory protein (MIP)-1β-induced tumor necrosis factor (TNF)-α and MIP-1α. It is regulated upon activation of normal T-cell expression and secretion (RANTES) in CIA rats. Additionally, serum levels of interleukin (IL)-1β, IL-2, IL-6, TNF-α, MIP-1α, MIP-2, RANTES, and interferon-inducible protein (IP)-10 are reduced in WTD-treated CIA rats, compared to non-treated CIA rats, suggesting that WTD might present with anti-inflammation effects via inhibiting release of inflammatory factors [18]. WTD has the ability to downregulate DNA methylation levels, while upregulating histone modification levels in RA patients [19]. DNA methylation levels are reduced while H3 acetylation levels of peripheral blood mononuclear cells (PBMCs) are increased in WTD-treated CIA rats, compared to that of non-treated CIA rats [19]. DNA methylation and histone modification are involved in the pathogenesis of RA, indicating that WTD might also display anti-inflammation effects through inhibiting DNA methylation, while promoting histone modification [20-23]. Consequently, the anti-inflammation proprieties of WTD, in combination with MTX, produced better therapeutic effects for RA, compared to that of oral MTX alone, in the present study. Results showed a reduction of DAS28 scores, ESR levels, and HAD-DI scores, as well as an elevation of LDA rates and overall response rates.

A commonly applied drug for RA treatment, MTX has been shown to have some typical AEs, such as infections, abnormal hepatic function, and neutrophil count reduction [24-26]. Unfortunately, there are no studies regarding the safety of WTD in RA patients until now. To assess the safety of oral WTD in RA patients, the current study collected the AEs of oral WTD, in combination with MTX, and compared it with oral MTX alone. Results revealed that no differences existed in AEs rates between the WTD &MTX group and MTX alone group, indicating that oral WTD combined with MTX was equally tolerated with oral MTX alone.

There were several limitations to the present study: (1) The numbers of patients recruited in the study was relatively small, which might have

decreased statistical efficiency; (2) The efficacy of WTD in established RA patients and inactive RA patients was not assessed in the current study; (3) The efficacy and safety of WTD, combined with MTX, in long-term medications, were not evaluated in this study.

In conclusion, WTD in combination with MTX is more effective and equally tolerated, compared to MTX alone, in treating early active RA patients.

Acknowledgements

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Disclosure of conflict of interest

None.

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