Plant Archives Vol. 21, Supplement 1, 2021 pp. 2168-2178 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
doi link : https://doi.org/10.51470/PLANTARCHIVES.2021.v21.S1.357

A SYSTEMATIC ANALYSIS ON A POTENTIAL ANTI-INFLAMMATORY AND IMMUNOSUPPRESSANT HERB *TRIPTERYGIUM WILFORDII* FOR THE MANAGEMENT OF RHEUMATOID ARTHRITIS

Tashi Tenzin, Amrinder Kaur* and Manish Vyas
Department of Ayurveda, School of Pharmaceutical Science, Lovely Professional University, Punjab, India.
*Corresponding author E-mail: amrinder.kaur@lpu.co.in

ABSTRACT

Rheumatoid arthritis (RA) is the systemic autoimmune and inflammatory disease. It is marked with swollen and tender joints, redness, pain and loss of physical functions. This mostly effects women than men and mostly prevalent in the age group of 35-50 years. *Tripterygium wilfordii* Hook F (TwHF) is the Chinese medicinal herb used in the Traditional Chinese Medicine (TCM) for treating various autoimmune and inflammatory diseases like rheumatoid arthritis (RA). A diterpenoid, triptolide is reported to be the major constituent in TwHF which is responsible for the therapeutic efficacy and the toxicity. This systemic analysis focuses on comparing and analysing the clinical efficacy and adverse events of TwHF extract used for the treatment of RA. Numerous preclinical studies (in vivo & in vitro) reported anti-inflammatory and immunosuppressant effect of TwHF extract in the RA models. TwHF extract is assumed to exhibit its anti-inflammatory and immunosuppressant effect by inhibiting the expression of proinflammatory cytokines such as IL-2, IL-6, IL-8, IFNγ, TNFα. It also works by inhibiting the expression of inflammatory mediators like COX-2, PGE_2, MMP1, MMP3, ICAM1, VCAM1. It induces apoptosis in lymphocytes and synovial fibroblast and inhibits the proliferation of B and T cells. In this analysis, results of the various clinical trials conducted by different researchers are reported and analysed for its clinical efficacy in RA. All most all the study reported significant improvement in arthritic score, swollen and tender joints, morning stiffness, DAS28, ESR and CRP level. Several studies and meta-analysis reported that TwHF extract/preparations are not inferior to DMARDs (MTX, Sulfasalazine) according to ACR20%, ACR50%, ACR70%, cDAI response and EULAR good response. In addition, it was reported that TwHF plus MTX combination therapy was superior and safe compared to MTX monotherapy.

This analysis has concluded that the studies included in this analysis have its own limitations as most of the clinical trials were conducted in China only (selection bias) and duration of trials were short. Whereby long-term efficacy, safety and toxicity was not known. Therefore, more clinical trials in future with longer follow-up period are required to warrant the clinical efficacy in the management of RA.

Keywords: *Tripterygium wilfordii,* Rheumatoid arthritis, Triptolide, Cyclooxygenase-2, DMARDs

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune and inflammatory disease (Chen *et al*., 2013). It is associated with swollen and tender joints, loss of physical function, pain and redness in the affected areas (Scott *et al*., 2010). And additional organ systems like pulmonary, cardiovascular, ocular, and cutaneous systems are also said to be involved in RA. However, in the clinical diagnosis of RA, the presence of inflammatory arthritis (IA) in the synovium is considered as the hallmark (Deane & Holers, 2019).

Worldwide, the annual incidence of RA is estimated to be 3 cases per 10,000 people (Singh *et al*., 2015). Approximately, 1.0 % of the world population lives with active RA (Hu *et al*., 2019). It mostly affects people in the age group of 35-50 years. And studies reported that it mostly affects women than men(Hu *et al*., 2019). According to World Health Organization (WHO) more than 23 million people in the world live with RA. RA is assumed to be the result of an immune response in which the body's immune system attacks its own healthy cells assuming it as the antigen. However, the specific cause and the origin of the disease is still unknown. And there is no permanent cure for RA till now but modern drugs like Disease Modifying Anti-Rheumatoid Drugs (DMARD's) and Non-steroidal Anti-inflammatory Drugs (NSAIDs) are used for the management of RA (Mota *et al*., 2013). Commonly used DMARD's like methotrexate, sulfasalazine and hydroxychloroquine are used to subside the symptoms and manage RA (Mota *et al*., 2013). However, it is associated with some severe side effects like GIT disturbances, stomach upset, bone marrow suppression, nausea,hair loss, diarrhoea, mouth sores, rashes and serious skin infection and lung, liver and kidney problems (Ferreira *et al*., 2016; Goulet *et al*., 2018). This systematic analysis/review focuses on the traditional Chinese herb *Tripterygium wilfordii*, a member of Celastraceae family

(known in china as “lei gong teng” or “thunder god vine”) which is used in the traditional Chinese medicine (TCM) for the treatment of joint pains, fever, chills, oedema, autoimmune diseases and local inflammation (Bao & Dai, 2011). It is found to be grown in the east and south of China, Japan, Myanmar, Vietnam and Korea (Liu *et al*., 2018). The chloroform/methanol extract (T2) and ethyl acetate (EA) extract of the roots of *Tripterygium wilfordii* Hook F (TwHF) have been investigated as a potential treatment for RA and other malignancies(Tao & Lipsky, 1995). In China, TwHF is approved for its usage in treating RA. While in the west the used of TwHF was prohibited and was only used as the pesticide. Recently, extracts of TwHF have also been tested in the West, with good efficacy for the treatment of the RA (Tao *et al*., 2002). TwHF has exhibited multiple pharmacological activities, such as anti-inflammatory, immune modulation, antitumor, and antifertility activities. Specifically, regarding its use for RA treatment, numerous preclinical studies have demonstrated immune-suppressive, anti-inflammatory and cartilage protective activities(Zhen *et al*., 1987; Brinker *et al*., 2007; Qui & Kao, 2003). The clinical application of TwHF extract and preparations (TwHF tablets, TwHF glycoside tablets and capsules) are limited and are not popular in the treatment of RA due to its toxicity (Tao, 1997). The most common side effects of TWHF are GIT disturbances, leukopenia, thrombocytopenia, rash, skin pigmentation, amenorrhea and dysfunctions of the male and female reproductive system (Tao & Lipsky, 2000). However, the carefully extracted extract of root of TwHF has exhibited significant clinical efficacy in the management of RA like that of modern DMARD’s i.e., methotrexate, sulfasalazine etc. (Liu *et al*., 2018; Goldbach-Mansky *et al*., 2009; Lev *et al*., 2015).

Botanical source

Common name: Thunder God Vine/lei-gong-teng

Botanical Name: *Tripterygium wilfordii*

Family: Celastraceae

Part used: Extract of root (other parts of this plant are poisonous in nature)

Botanical description

Thunder god vine is the translated English name of the perennial plant *lei gong teng* (Chinese name). In Asia, the plant is also known by the name "three-wing-nut”. It is a deciduous climbing vine which shades its leaves. It produces white flowers and red fruit with three "wings”. Other than root (excluding root bark), plant’s leaves, flowers, and outer skin of the root are poisonous (Jiang & Zhao, 1987; Chen *et al*., 1987). Accounting to the poisonous nature of plant, in the west it was used as pesticide and its use for the medicinal purpose was prohibited (Tao, 1997).

However, its root pulp is the non-poisonous part, which is used in china for medicinal purpose and gradually in late 20^{th} century used of root extract for RA management came to light in the west (Tao *et al*., 2002; Goldbach-Mansky *et al*., 2009). Owing to highly poisonous nature of plant, it is referred by the two Chinese folk names "Walk seven steps and die" and "Intestine-breaking plant.” InChina, traditional practitioners carefully extracted the portion of *lei gong teng* used for treatment. The root portion of the plant was collected in summer/early fall. In past, root of the plant is powdered and applied tropically over the affected area as to avoid the toxic effect upon the oral administration.

Chemical Constituents

Numerous studies have reported that TwHF contains more than 70 different constituents, including triterpenes, diterpenes, glycosides, alkaloids and 95% of them are terpenoids (Tao & Lipsky, 2000, 22-24, Zhang *et al*., 1990; Zheng *et al*., 1987). The three major diterpenoids; triptolide, tripdiolide, and triptonide (Zheng *et al*., 1987; Chen, 2001)are the most abundant and accredited for the immunosuppressive and anti-inflammatory effects exhibited by the root extracts in both in vitro and in vivo studies (Zhen *et al*., 1987; Brinker *et al*., 2007; Qui & Kao, 2003).

Most of the studies concluded that diterpene triepoxide, triptolide is the major active constituent of the *Tripterygium wilfordii* (Zheng *et al*., 1987). The compounds which exhibit similar activities like that of triptolide such as tripdiolide, 16-hydroxytriptolide, triptonide, tripchloride and triptriolide are also found in the extract of TwHF (Tao & Lipsky, 2000).

However, the active ingredient of TwHF is still not clear but researchers and investigators believe that triptolide and its related compounds are responsible for its therapeutic effects and toxicity (Zheng *et al*., 1987). One this account, the content of this component has been used to standardize the ethyl acetate (EA) extract in China. Table 1 describes the major constituent found in TwHF extract.

Probable mechanism of action

Effects on proinflammatory mediators

The potent inhibitory effect of the inflammation of TwHF was observed in the carrageenan-induced paw oedema, croton oil-induced ear swelling and carrageenan-stimulated air pouch model of the inflammation in animals (Qui & Kao, 2003; Tao, 1997). It was observed that the animals treated with the root extract of TwHF had significantly lower counts of white blood cell (especially decrease in neutrophils counts), lower volume of air pouch exudate, and lower concentration of prostaglandin E_2 (PGE_2), nitrite and tumour necrosis factor (TNF) in the exudate of the experimental animals. TwHF selectively inhibits the expression of cyclooxygenase 2 (COX-2) without inhibiting the expression of COX-1 in the non-inflamed tissue/organ. Which thereby selectively inhibits the PGE_2 productions in the inflamed/injured tissue or organ (Qui & Kao, 2003; Tao *et al*., 1999). Which otherwise not inhibited will cause the pathogenesis of RA. TwHF extract also exhibited inhibitory effect of the PGE_2 production by lipopolysaccharide-induced animal model. Whereby indicating the suppression of COX-2 without interfering the expression of COX-1. Besides the inhibition of COX-2, it was also found to inhibit the transcription of the inducible nitric oxide synthase (iNOS) gene there by inhibiting the production of nitric oxide and it was also found to block messenger ribonucleic acid (mRNA) transcription thus inhibiting the production of matrix metalloproteinases 3 (MMP-3) and matrix metalloproteinases 13 (MMP13) (Tao *et al*., 1998; Guo *et al*., 2001; Sylvester *et al*., 2001). Triptolide one of the main components of TwHF also shows the similar effect to that of TwHF extract. In which it also suppresses the expression of COX-2 and the precursor forms of MPP-1 and 3 and thereby inhibiting the production of PGE_2, NO and lipoxygenase (Tao *et al*., 1998; Lin *et al*., 2001). Nuclear factor Kappa B (NF-kB) which

activates the transcription of genes for iNOS and COX-2 is inhibited by TwHF extract and triptolide i.e., it inhibits the binding of NF-kB to the DNA (Sylvester *et al*., 2001).

Effects on proinflammatory cytokines

Cytokines are regulators of host responses to infection, immune responses, inflammation, and trauma. Aproinflammatory cytokine is a type cytokine which promotes systemic inflammation. And the proinflammatory cytokines produced by lymphocyte and macrophages in the RA infiltrates into synovium and causes severe inflammation to the host. Studies indicated that the extract of TwHF supresses the production of proinflammatory cytokines like TNFα, interleukin 2 (IL-2), IL-6, IL-7 and interferon gamma (IFN-γ) by T lymphocytes and macrophage in response to the foreign material (antigen) (Chen, 2001; Luk *et al*., 2000). Similarly, triptolide also exhibited inhibition of T-cell proliferation and production of TNFα, IL-1, IL-2, IL3, IL-4, IL-5, IL6, and IL-8 by the stimulated T cell (Sylvester *et al*., 2001; Lin *et al*., 2001; Zhou *et al*., 2003). Triptolide was also found to inhibit the lipopolysaccharide (LPS) induced dendritic cell production of proinflammatory proteins (Liu *et al*., 2006).

Effects on adhesion molecules

RA is associated with the inflammation of the synovial joints. During inflammation, adhesion molecules causes the attraction of T-cells to the site of inflammation. And the extract of TwHF had shown to exhibit the inhibition of secretion and expression of vascular cellular adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) by stimulated human synovial fibroblast thereby reducing the inflammation in the synovial joints(Zhou *et al*., 2003; Chang *et al*., 1999).

Effects on humoral immune response

In vitro studies reported that the extract of TwHF extract inhibited the proliferation and production of immunoglobulins by pokeweed mitogen-stimulated peripheral blood mononuclear cells or purified human B cells in response to stimulation with *Staphylococcus aureus* (SA) (Lee *et al*., 1995; Ye *et al*., 1990). Whereby exhibiting that TwHF can affect function of B-cells like that of T-cells. One study also reported that the TwHF extract inhibited the formation of antibody against the sheep red blood cell (SRBC) in the mice(Tao *et al*., 1999). It was also found that the administration of TwHF extract reduces the production of igM and igM-rheumatoid factor by pokeweed mitogen stimulated peripheral blood mononuclear segregated from the patients(Tao *et al*., 1988).

Effects on cell proliferation

TwHF extract induces the apoptosis of T or B cells, reduce inflammation triggered by these cells and strongly inhibit the proliferation of T-cells and B-cells (Tao *et al*., 1998; Ho *et al*., 1999; Li & Weir, 1990). Similarly, triptolide was found to inhibit proliferation of B and T cells (Tong *et al*., 1999). Thereby making the TwHF extract as the potential alternative solution for the management of RA alongside the modern synthetic DMARDs and NSAIDs.

Effects on synovial fibroblast and chondrocytes

Rheumatoid arthritis synovial fibroblasts (RASF) are associated with excessive activation and anapoptosis-resistantphenotype, leading to enlargement (hyperplasia) of the synovium (Klein *et al*., 2012). RASF has the ability to produce inflammatory cytokines and matrix degrading molecules such as MMPs (i.e., MMP-3 and MPP-13), and thereby promoting inflammation and degrading the joints causing inflammation, redness, pain, disability, swelling and loss of function. The production of matrix metalloproteinases (MMPs) leads to invasion of RASF into cartilage (Klein *et al*., 2012). Studies reported that the TwHF extract inhibits the PGE_2 production by IL-1 stimulated RASF in the dose-dependent manner and it was also reported to supress COX-2 mRNA expression there by inhibiting the PGE_2 production(Zhou *et al*., 2007). On combination therapy, both TwHF and triptolide decrease the number of RASFs by inducing apoptosis, inhibit the function of RASFs to produce PGE2, proinflammatory cytokines and MMPs and inhibit its proliferation. Studies also reported that the chondrocyte itself destroys the cartilage by production of MMPs. Both TwHF extract and triptolide had shown to inhibit mRNA and protein expression of cytokine induced MMP-3 and MMP-13 in stimulated chondrocytes by impairing NF-κB binding activities and activating protein-1 (AP-1)(Sylvester *et al*., 2001; Liacini *et al*., 2005).

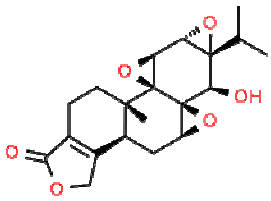
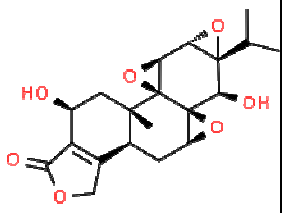
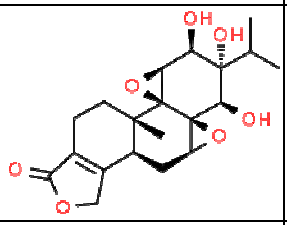
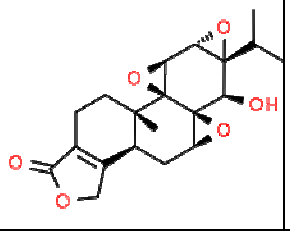
Pharmacological activities

Both in vivo and in vitro studies of TwHF extract reported the anti-inflammatory and immunosuppressant activity in RA models (Zhen *et al*., 1987; Brinker *et al*., 2007; Qui & Kao, 2003). Other pharmacological activities such as immune modulation, antitumor, antifertility and anti-HIV effects are also reported in the studies (Zhen *et al*., 1987; Brinker *et al*., 2007; Qui & Kao, 2003; Chen *et al*., 1992; Duan *et al*., 1999). In this systematic analysis emphasis are given to anti-inflammatory and immunosuppressant activity of TwHF extract/preparations.

Anti-inflammatory activity

A numerous in vivo study has reported that components of TwHF significantly suppress inflammatory responses in rheumatoid arthritis. Administration of TwHF extract in the collagen-induced arthritis mice and the adjuvant-induce arthritis rat showed marked reduction in the inflammation and lowered arthritic score (Dai *et al*., 2013; Wang & Xie, 1999). Whereby indicating that TwHF has some anti-inflammatory effect. Treatment with TwHF extract lowered the expression levels of receptor activator of NF-κB ligand (RANKL) in synovium, subchondral, and trabecular bone of rats with adjuvant-induced arthritis. NF-κB, otherwise activates RASF to bind DNA thereby causing destruction of cartilage and joints. Severity and the occurrence of the arthritis were significantly reduced in the CIA mice even if the TwHF extract was administered 3 weeks after the first introduction of collagen in the mice. Which thereby suggest the high therapeutic efficacy of TwHF (Klein *et al*., 2012). In vivo anti-inflammatory and immunosuppressant activities of TwHF are given in Table 3.

Table 1 : Major chemical constituents and its related components of TwHF extract.

Sl.no.	Part used (Extract)	constituents	Pharmacological activities	Structure	Ref.
1.	Root	Triptolide	Anti-inflammatory & immunosuppressant effect		(Zheng *et al*., 1987; Chen, 2001)
2.	Root	Tripdiolide	Anti-inflammatory & immunosuppressant effect		(Zheng *et al*., 1987; Chen, 2001)
3.	Root	Triptriolide	Anti-inflammatory & immunosuppressant effect		(Zheng *et al*., 1987; Chen, 2001)
4.	Root	PG490 (pure triptolide)	Anti-inflammatory & immunosuppressant effect		(Zheng *et al*., 1987; Chen, 2001)

Immunosuppressant activity

Previous studies performed in China noted that extracts of TwHF suppressed activities of mouse and rat lymphocytes (T cells and B cells proliferation) in vitro (Asano *et al*., 2000; Yu *et al*., 2017). In vivo studies also showed that the extracts of TwHF inhibit transcription of a variety of genes involved in promoting immune and inflammatory responses by a novel mechanism (Tao, 1997).

Both the root extracts of EA and T_2 contain triptolide and tripdiolide as the major immunosuppressive diterpenoids at varying concentrations. And immunosuppressant activity of the EA extract was accounted for triptolide. While both triptolide and tripdiolide along with other components were necessary for the inhibitory effect of T_2 (Tao & Lipsky, 1995). In vivo anti-inflammatory and immunosuppressant activities of TwHF are given in Table3.

Effect of T_2 on human T cell responsiveness

Study carried out by Tao.et.al to evaluate the effect of T2 on human T cell responsiveness found out that the IL-2 production by T cells stimulated by either phytohemagglutinin (PHA) or anti-CD3 was significantly reduced by T2. However, these results suggested that the IL-2-stimulated pathway of T cell proliferation is not inhibited by T2 (Tao *et al*., 1991).

Effect of T_2 on human B lymphocyte responses

T_2 alsoinhibited proliferation of B cells stimulated by *Staphylococcus aureus* (SA) or anti-CD3(49). It was also noted that the addition of IL-2 did not reverse the T_2-mediated inhibition of anti-CD3-activated T cell-dependent B cell proliferation (Tao *et al*., 1991).

Effect of T2 on monocyte function

Many previous studies concluded that T2 inhibits the T cell proliferation in vitro. Current analysis also found out that culturing of antigen with the monocyte in the presence of T2 significantly inhibits the proliferation of T-cell and the concentration as low as 0.08µg/ml was enough to inhibit its proliferation in the culture (Tao *et al*., 1991).

Similarly, triptolide also exhibited its ability to inhibit inflammation and supress immune system in the RA models. However, studies found out that triptolide do not produce the same therapeutic efficacy at the concentration equal to that of EA extract and T2. Whereby requiring more concentration than that of T2 to produce same therapeutic response (Tao *et al*., 1991).

Table 2 : Effect of TwHF extract on the proinflammatory mediators or signalling molecules

Sl. No.	Molecules (Mediators/cytokines /receptors/Ig)	Cell sources	Extract	Stimuli	Effect	Ref.
1	COX-2	Human synovial cells	Ethanol extract	IL-1β	Inhibition	(Maekawa *et al*., 1999)
2	MMP1/MMP3	Human monocytes / Human synovial fibroblasts	T2 or triptolide	LPS	Inhibition	(Zhang *et al*., 2008; Luk *et al*., 2000)
3	PGE2	Human monocytes	T2 or triptolide	LPS	Inhibition	(Zhang *et al*., 2008)
4	ICAM-1	Human synovial fibroblasts	Triptolide	LPS	Inhibition	(Luk *et al*., 2000)
5	IgA, IgM, IgG	Human B cells	Triptolide	SA + IL-2	Inhibition	(Tao *et al*., 1991)
6	IL-2 receptor	Mouse spleen cells	Triptolide	ConA or PHA	Inhibition	(Si-Xun *et al*., 1994)
7	IFNγ	Human T cells	Triptolide	PMA/PHA	Inhibition	(Chan *et al*., 1999)
8	TNFα	Mouse macrophage	Triptolide	LPS	Inhibition	(Luk *et al*., 2000)
9	IL-2	Human PBLs	Triptolide	PMA/iono mycin	Inhibition	(Qui *et al*., 1999)

Abbreviations: ICAM-1: intercellular adhesion molecule 1; PBLs: peripheral blood lymphocytes; ConA: concanavalin A; PHA: phytohaemagglutinin; PMA: phorbol 12-myristate 13-acetate; SA: *Staphylococcus aureus*; LPS: lipopolysaccharide

Table 3 : Showing in vivo anti-inflammatory and immunosuppressant effect of TwHF extract

Sl. No.	Model	Animal	Extract	Part used	Results	Ref.
1	Croton oil-induced ear swelling	Mouse	Triptolide	Root	Reduce inflammation	(Dai *et al*., 2013)
2	Antibody production to SRBC	Mouse	Ethyl acetate	Root	Inhibits the formation of antibody against SRBC	(Wang &Xie, 1999)
3	Antibody production to SRBC	Mouse	Triptolide	Root	Inhibits the formation of antibody against SRBC	(Dai *et al*., 2013)
4	Adjuvant arthritis	Rat	Ethyl acetate	Root	Reduce inflammation, lowers arthritic score	(Wang &Xie, 1999)
5	Skin graft	Mouse	Chloroform/methanol(T2)	Root	Immunosuppressant effect	(Qui *et al*., 2017)
6	Collagen-induced arthritis	Rat	Triptolide	Root	Reduce inflammation, lowers arthritic score	(Gu *et al*., 1998)
7	Allergic encephalomyelitis	Guinea pig	Chloroform/methanol(T2)	Root	Reduce inflammation	(Cheng, 1985)
8	Cardiac allograft	Rat	Triptolide (PG490)	Root	Immunosuppressant effect	(Hachinda *et al*., 1999)
9	Renal allograft	Rat	Triptolide (PG490)	Root	Immunosuppressant effect	(Wang *et al*., 2000)
10	Nephritis	Rabbit	Ethyl acetate	Root	Reduce inflammation in kidney	(Mao &Haung, 2016)
11	Chronic GVHD	Mouse	Chloroform	Root	Immunosuppressant effect	(Asano *et al*., 1997)

Abbreviations: SRBC: sheep red blood cell; GVHD: graft versus host disease

Clinical studies

Numerous in vivo and in vitro studies have reported anti-inflammatory and immunosuppressant activity of the root extract of *Tripterygium wilfordii* (Zhen *et al*., 1987; Brinker *et al*., 2007; Qui & Kao, 2003). Owing to its anti-inflammatory activity, this particular plant has been used in china for the treatment of RA. So, to validate its efficacy in RA management numerous human clinical trials of TwHF extract was conducted. Many of the human trials are reported from china and three was reported from United States (US). Many trials have claimed about its therapeutic efficacy in RA management.

First ever prospective, double-blind, crossover human clinical trial of chloroform/methanol extract (T_2) of TwHF was conducted by Tao *et al*. in 1989. In that clinical trial, total of 70 active RA patients are randomly divided in to groups; group A and group B. The study was conducted for the total duration of 16 weeks. In that study patients in group A was treated with T_2 extract, 60mg per day for the 12 weeks. And the patient who have successfully completed 12 weeks of T_2 extract was then treated with placebo for subsequent 4 weeks. When it comes to group B. Active RA patient in this group are first administered with placebo for duration of 12 weeks and the patient who have successfully completed 12 weeks placebo treatment were then treated with the T_2 extract for subsequent 4 weeks. In Group A, 27 out of 35 RA patients completed 12-week T2 treatment and of 27, 3 withdrew from the trial owing to the adverse events and non-medical reasons and only 24 completed the subsequent 4-week placebo treatment. While in the group B, 31 of out 35 RA patients completed the 12-weeks of placebo treatment and 4 patients withdrew from the trial owing to adverse events and side effects and only 27 completed the subsequent 4-week T2 treatment.

Per protocol (PP) analysis found out that Group A showed significant decrease in the tender joints count, swollen joints count, duration of morning stiffness,

erythrocyte sedimentation rate and C-reactive protein. And significant improvement in the 15 m walking time, mean grip strength, IgA, IgG, IgM and significant improvement in physician's and patient's and global assessment compared to the group B after the completion of 12 weeks of treatment. Which indicated that the T_2 extract is more effective in RA management than placebo. The common side effect associated with patients receiving T_2 treatment were gastrointestinal tract disturbances (diarrhoea), amenorrhea (due to cumulative dose of T_2), vomiting, leukopenia, thrombocytopenia, oral ulcer, skin pigmentation and anorexia. Adverse events during T_2 treatment in RA patients are given in table 4.

In another, double-blind, placebo-controlled study conducted by Tao *et al*. in 2002 in the Parkland Memorial Hospital, Dallas, Texas, USA to assess the safety and therapeutic efficacy of root extract of TWHF in RA, 35 patients were randomly divided into high dose group (11 people, 360mg/day), low dose group (12 people, 180mg/day) and placebo group (12, 180mg/day) receiving the treatment for 20 weeks followed by the assessment of long term tolerability, the open label extension and the therapeutic efficacy criteria of disease improvement by 20% of American college Rheumatology were being followed. The study result observed that 80% (8 of 11) from high dose group and 40% (4 of 12) from low dose group fulfilled 20 % disease improvement criteria but no one from placebo group accomplished these criteria. 5 patients (50%) from high dose group and 1 patient (10%) from low dose group were observed to have achieved 50% disease improvement criteria, while only 1 patient from high dose group met 70% disease improvement criteria. Shown in figure 1.

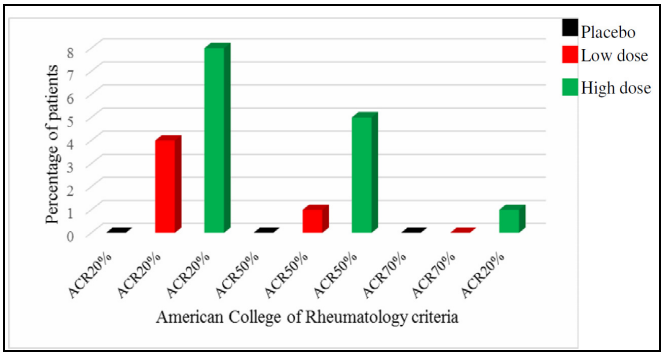


Fig. 1 : Graph showing the response of treatment to ACR20%, ACR50%, ACR70% improvement criteria in 3 groups-placebo, low dose and high dose group (Data from Tao *et al*., 2002)

The study result also shows that high dose group achieved the 20 % disease improvement criteria rapidly than low dose group with mean duration of 7 weeks and 12 weeks for high dose group and low dose group respectively. Significant decrease in number of swollen joints, tender joints, improvement in physician and patient's global assessment of disease activity and patient rated physical function and significant improvement in ESR and CRP were observed in high dose group. In high dose group the duration of morning stiffness decreased from baseline 145 minutes to 37 minutes and 26 minutes at 4 weeks and 20 weeks respectively. And the significant decrease in rheumatoid factor (RF) titer was observed in high dose group. While there was some degree of decrease in number of swollen and tender joints in low dose group compared to the placebo group.

Whereby indicating that high dose TwHF extract was effective than low dose and placebo. But low dose was significantly effective than placebo.

During that treatment adverse events are noticed in all the groups. The most common adverse event/side effect was diarrhoea. While other side effects like hair loss, nausea and headache was also seen in the patients receiving the treatment. Detailed side effects of patient in high dose, low dose and placebo are given in the table 5.

In most of the clinical trials, TwHF was orally administerd. But in a randomized, double-blind, placebo-controlled trial conducted by Thorne A *et al*. in 2003, TwHF in the form of tincture was tropically applied up to 6 times a day on the swollen and tender joints. After 6 weeks of daily application of tincture of TwHF. It was reported that tropical application of TwHF tincture was superior to placebo in reducing the RA activity.

Another study was carried out by Kempf P *et al*. in 2009 in US to compare the therapeutic efficacy of TwHF extract with that of sulfasalazine 2g/day (approved standard dose for RA) for the duration of 6 months. In that study it was observed that TwHF extract had rapid improvement in clinical signs and symptoms such as joint swelling, joint pain, CRP, ESR, IL-6 and decrease in rheumatoid factor (RF) in RA patients TwHF exhibited greater improvement than sulfasalazine (2g/day) in terms of patient achieving ACR20, ACR50, ACR70 response. The therapeutic efficacy of TwHF was significantly apparent after 2 weeks of the treatment while in the sulfasalazine treated group the effect and in the TwHF group than sulfasalazine group. The study concluded that TwHF has rapid and greater improvement than sulfasalazine in management of RA activity.

Table 4 : Adverse events during T_2 treatment in RA patients

Adverse events	First Treatment				Second Treatment			
	Group A (n=35)		Group B (n=35)		Group A (n=27)		Group B (n=31)	
	Case	%	Case	%	Case	%	Case	%
Rash	15	43	1	3	0	0	7	23
Anorexia	2	16	0	0	0	0	0	0
Abdominal pain	2	6	1	3	0	0	0	0
Diarrhoea	6	17	0	0	0	0	2	6
Amenorrhea	5	31	0	0	5	31	1	6
Postmenopausal vaginal bleeding	1	10	0	0	0	0	0	0
**n=total number of patients in treatment. Data from Tao *et al*.1989.								

In another multicentre randomised open-label extension trial conducted by Lv Q-wen *et al*. in 2014, 207 active RA patients were assigned randomly into three groups; 69 in MTX monotherapy group, MTX 12.5 mg/week, 69 in TwHF monotherapy group, TwHF 20 mg three times/day and 69 in combination therapy group. The study analysis indicated that 76.8% (53 of 69), 55.1% (38 of 69) and 46.4% (32/69) from combination therapy, TWHF monotherapy and MTX therapy respectively attained 50% disease improvement criteria of American college of rheumatology at 24 weeks.

A significant ESR reduction was achieved in the TwHF and the combination groups at week 12, whereas in the MTX group a significant reduction in ESR was not seen until week 24. At week 4, the TwHF group had greater improvement than the MTX group. TwHF monotherapy group and combination group achieved significant reduction in ESR at week 12 while MTX group significant reduction in ESR was not achieved until week 24. TwHF group exhibited greater improvement than MTX group in terms of ESR at week 4. Whereas MTX's effect was not shown until 24 weeks. Indicating that TwHF is not inferior to MTX monotherapy.

While adverse effects were observed in about 52.7% of patients of all the group and the common adverse outcome being gastrointestinal events in 34.8%, 43.5%, and 29.0% in combination therapy, MTX monotherapy and TwHF monotherapy respectively but these gastrointestinal adverse events were mild. This particular study concluded that combination therapy is better than MTX monotherapy in disease improvement, management of RA and safety. The study also reported that TwHF monotherapy is not inferior to MTX monotherapy.

Table 5 : Side effects of patient in high dose, low dose and placebo group.

Sl. No.		Placebo (12/35)	TwHF extract	
			Low dose (12/35)	High dose (11/35)
1	Diarrhoea	-	4	3
2	Headache	2	1	1
3	Nausea	-	2	1
4	Blister	1	-	2
5	Hair loss	-	2	1
6	Flatulence	1	-	1
7	Constipation	1	1	1
8	Tinnitus	-	-	1
9	Vaginal spotting	-	-	1
10	Heart burn	-	2	-
11	Facial rashes	-	-	1
12	Indigestion	1	-	1
13	Abdominal pain	-	-	1
14	Total no. of patients with ≥ 1 adverse event	4	6	5

***TwHF extract i.e., Ethyl acetate (EA) extract is used in this experiment.
Data taken from Tao *et al*., 2002

Adverse Events

Other than root, all other parts of this plant is highly poisonous and toxic in nature (Jiang & Zhao, 1987; Chen *et al*., 1987). However, even the root extract of TwHF is associated with side effects. The common side effects of patient receiving the TwHF treatment are GIT disturbances, diarrhoea, thrombocytopenia, rashes, skin pigmentation, male infertility, dysmenorrhea and amenorrhea (Tao & Lipsky, 2000). Due to this side effect, TwHF treatment is recommended only to the post-menopausal women with RA. Adverse event due to the TwHF administration in RA patients are given in the table 4 and 5.

Discussion

Tripterygium wilfordii is the herb used in Traditional Chinese Medicine (TCM) for the treatment of various autoimmune and inflammatory diseases. In China TwHF extracts/preparation along the MTX had been approved to be used in treating active RA patients (Lev *et al*., 2015). In addition, various clinical trials conducted in China and 3 in US (Tao & Lipsky, 2000; Goldbach-Mansky *et al*., 2009; Tao *et al*., 2001) reported about the therapeutic efficacy of TwHF in the management of RA. A diterpenoid, triptolide is reported to be the major constituent in TwHF which is responsible for the therapeutic efficacy and the toxicity (Zheng *et al*., 1987).

And the anti-rheumatic activity of TwHF and triptolide (main constituent) is exhibited by inhibiting the expression of proinflammatory cytokines such as IL-2, IL-2 receptor, IL-6, IL-8,IFNγ, TNFα (Maekawa *et al*., 1999; Zhang *et al*., 2008; Luk *et al*., 2000; Tao *et al*., 1991; Si-Xun *et al*., 1994; Chan *et al*., 1999 Qui *et al*., 1999). It also works by inhibiting the expression of inflammatory mediators like COX-2, PGE_2, MMP1, MMP3, ICAM1, VCAM1. Studies reported that it works by inducing apoptosis in lymphocytes and synovial fibroblast. It also inhibits the proliferation of B and T cells (immunosuppressant effect) (Klein *et al*., 2012; Tao *et al*., 1991) and supress the synovial fibroblast and chondrocytes from producing MMPs which causes the degradation of joints and cartilage.

In the modern medicine system, NSAIDs, DMARDs and biological agents are mostly used in the management of RA (Hu *et al*., 2019; Lev *et al*., 2015). However, they don't provide permanent cure to RA but they just provide temporary relieve from the pain and symptoms. Administration of NSAIDs and DMARDs are associated

with severe adverse events like GIT disturbances, diarrhoea, rashes, hepatotoxicity, cardiac abnormalities, blood dyscrasias, lung diseases etc (Ferreira *et al*., 2016; Goulet *et al*., 2018). All the aforementioned therapy inhibits both COX-1 and COX-2. Due to the inhibition of COX-1, essential physiological function of the body gets disturbed and as a result there is stomach upset, intestinal bleeding and ulcers in the stomach. While studies have reported that the TwHF extract only inhibits COX-2 without inhibiting COX-1(Tao *et al*., 1999). Whereby making it a better, safe and cost-effective alternatives in the management of RA.

Numerous in vivo and in vitro studies conducted for TwHF extract reported immunosuppressant and anti-inflammatory effect on various RA models (Zhen *et al*., 1987; Brinker *et al*., 2007; Qui & Kao, 2003). Other therapeutic activities like immunemodulation, antitumor, antifertility and anti-HIV effects are also reported. All the clinical trials conducted in china and 3 in US reported the similar clinical efficacy and benefits of TwHF extracts/preparations in the management of RA patients. Recent meta-analysis conducted in the 2017 reported that TwHF preparation is superior to MTX monotherapy (according to ACR20, ACR50, ACR70) in the RA management. It also reported that the DMARDs (i.e. MTX) showed frequent and severe adverse events than TwHF preparations. Another study conducted in 2014 reported that TwHF monotherapy was not inferior to MTX monotherapy but combination therapy was superior to MTX monotherapy (Lev *et al*., 2015). It also concludes that combination therapy of MTX and TwHF is effective and safe as well. However, TwHF is also associated with adverse events like GIT disturbances, rashes, diarrhoea, dysmenorrhea and amenorrhoea etc (Tao & Lipsky, 2000).

All in vivo, in vitro and clinical studies reported the clinical efficacy of TwHF in RA, however the studies included in this analysis are associated with certain limitations. Most of the clinical trialswere conducted in China (only 3 in US) (Tao & Lipsky, 2000;Goldbach-Mansky *et al*., 2009; Tao *et al*., 2001) thereby resulting in selection bias (Chinese population). In different trials different concentration (dose) of TwHF extract was used, which makes it difficult to generate one concrete result and findings. Time period of all the clinical trials were short (some had 20 weeks, 24 weeks, 6 months). Owing to the short study duration, long term efficacy, safety and toxicity of TwHF extract/preparation was not known.

Due to severe side effects associated with modern synthetic drug and high cost, the focus is gradually shifting towards the herbal treatment. Therefore, to substitute TwHF extract and its preparation in management of RA. I would recommend future researchers and scholars to extend their duration of study/trials to evaluate long term efficacy, safety and toxicity associated with the particular therapy. And the proper standardization of clinical trial procedures, which will help future researcher to efficiently analyse and compare various trials conducted.

And this systematic analysis will provide future academicians, scholars and researchers, an overview of TwHF extract/preparations in the management of RA including its botanical description, major chemical constituents, probable mechanism of action, pharmacological studies, clinical studies and adverse events associated with it. Whereby making it easier for them to proceed with the further studies which were found lacking in this particular analysis.

Conclusion

This systematic analysis concluded that TwHF extract/preparation is effective in the management of RA with comparatively less toxicity than modern DMARDs like MTX and sulfasalazine. Even though TwHF monotherapy is effective, this analysis also concluded that MTX + TwHF combination therapy is superior to both TwHF and MTX monotherapy. However, to fully warrant its clinical efficacy in the management of RA, further studies with longer follow up periods are required to evaluate, analyse and examine the long-term efficacy, safety and toxicity associated with TwHF administration.

References

Asano, K.; Sugimoto, K.; Watanabe, T.; Hisano, M. and Hisamitsu, T. (2000). Suppressive effects of the Chinese herbal remedy Tripterygium wilfordii Hook f on eosinophilia and IgE hyperproduction in mice. Allergology International, 49(1): 37-45.

Asano, K.; Yu, Y.; Kasahara, T. and Hisamitsu, T. (1997). Inhibition of murine chronic graft-versus-host disease by the chloroform extract of Tripterygium wilfordii Hook f. Transplant Immunology, 5(4): 315-319.

Bao, J. and Dai, S. M. (2011). A Chinese herb Tripterygium wilfordii Hook F in the treatment of rheumatoid arthritis: Mechanism, efficacy, and safety. Rheumatology International, 31(9): 1123–1129.

Brinker, A.; Ma, J.; Lipsky, P. andRaskin, I. (2007). Medicinal Chemistry and Pharmacology of Genus Tripterygium (Celastraceae). Cheminform, 38(27).

Chan, M.; Kohlmeier, J.; Branden, M.; Jung, M. and Benedict, S. (1999). Triptolide is more effective in preventing T cell proliferation and interferon-gamma production than is FK506. Phytotherapy Research, 13(6): 464-467.

Chang, D.; Kuo, S.; Lai, J. and Chang, M. (1999). Effects of anti-rheumatic herbal medicines on cellular adhesion molecules. Annals of The Rheumatic Diseases, 58(6): 366-371.

Chen LN, Cai H, Yu DY. (1987). Clinical analysis of 10 cases of Tripterygium wilfordii Hook induced toxicity. Proc Syrup Clinical Application of Tripterygium wilfordii Hook. Hubei, China.

Chen, B. (2001). Triptolide, A Novel Immunosuppressive and Anti-Inflammatory Agent Purified from a Chinese Herb Tripterygium Wilfordii Hook F. Leukaemia& Lymphoma, 42(3): 253-265.

Chen, K.; Shi, Q.; Fujioka, T.; Zhang, D.; Hu, C. andJin, J. *et al*. (1992). Anti-AIDS Agents, 4. Tripterifordin, a Novel Anti-HIV Principle from Tripterygium wilfordii: Isolation and Structural Elucidation. Journal of Natural Products, 55(1): 88-92.

Chen, L.; Qi, H.; Jiang, D.; Wang, R.; Chen, A.; Yan, Z. and Xiao, J. (2013). The New Use of an Ancient Remedy: A Double-Blinded Randomized Study on the Treatment of Rheumatoid Arthritis. The American Journal of Chinese Medicine, 41(02): 263-280.

Cheng, H.W. (1985). Cellular immunological study on experimental allergic encephalopathy and the

exploration of the effect of Tripterygium wilfordii Hook on it. Immunol Bull 5:7. (Chinese)

Cibere, J.; Deng, Z.; Lin, Y.; Ou, R.; He, Y.; Wang, Z.; Thorne, A.; Lehman, A. J.; Tsang, I. K. and Esdaile, J. M. (2003). A randomized double blind, placebo-controlled trial of topical Tripterygium wilfordii in rheumatoid arthritis: reanalysis using logistic regression analysis. The Journal of rheumatology, 30(3): 465–467.

da Mota, L.; Cruz, B.; Brenol, C.; Pereira, I.; Rezende-Fronza, L. and Bertolo, M. *et al*. (2013). Guidelines for the drug treatment of rheumatoid arthritis. RevistaBrasileira De Reumatologia (English Edition): 53(2):158-183.

Dai, S.; Yin, K.; Yao, X. and Zhou, L. (2013). Inhibition of interleukin-13 gene expression by triptolide in activated T lymphocytes. Respirology, 18(8): 1249-1255.

Deane, K. D. and Holers, V. M. (2019). The Natural History of Rheumatoid Arthritis. Clinical Therapeutics, 41(7): 1256–1269.

Duan, H.; Takaishi, Y.; Bando, M.; Kido, M.; Imakura, Y. and Lee, K. (1999). Novel sesquiterpene esters with alkaloid and monoterpene and related compounds from Tripterygium hypoglaucum: A new class of potent anti-HIV agents. Tetrahedron Letters, 40(15): 2969-2972.

Ferreira, J.; Ahmed Mohamed, A. and Emery, P. (2016). Glucocorticoids and Rheumatoid Arthritis. Rheumatic Disease Clinics of North America, 42(1): 33-46.

Goldbach-Mansky, R.; Wilson, M.; Fleischmann, R.; Olsen, N.; Silverfield, J.; Kempf, P.; Kivitz, A.; Sherrer, Y.; Pucino, F.; Csako, G.; Costello, R.; Pham, T. H.; Snyder, C.; van der Heijde, D.; Tao, X.; Wesley, R. and Lipsky, P. E. (2009). Comparison of Tripterygium wilfordii Hook F versus sulfasalazine in the treatment of rheumatoid arthritis: a randomized trial. Annals of internal medicine, 151(4): 229–W51.

Goulet, J.; Buta, E.; Brennan, M.; Heapy, A. and Fraenkel, L. (2018). Discontinuing a non-steroidal anti-inflammatory drug (NSAID) in patients with knee osteoarthritis: Design and protocol of a placebo-controlled, noninferiority, randomized withdrawal trial. Contemporary Clinical Trials, 65, 1-7.

Gu, W. andBrandwein, S. (1998). Inhibition of type II collagen-induced arthritis in rats by triptolide. International Journal of Immunopharmacology, 20(8): 389-400.

Guo W, Ma L, Tao X. (2001). In vitro inhibitive effects of Tripterygium wilfordii on NO production, iNOS activity, and iNOS mRNA expression in chondrocytes of patients with rheumatoid arthritis. Zhonghua Yi Xue Za Zhi 81:1035–1037 (in Chinese)

Hachida, M.; Lu, H.; Zhang, X.; Saito, S.; Furutani, Y. and Matsuoka, R. *et al*. (1999). Inhibitory effect of triptolide on platelet derived growth factor-A and coronary arteriosclerosis after heart transplantation. Transplantation Proceedings, 31(7): 2719-2723.

Ho, L. J.; Chang, D. M.; Chang, M. L.; Kuo, S. Y. and Lai, J. H. (1999). Mechanism of immunosuppression of the antirheumatic herb TWHf in human T cells. The Journal of rheumatology, 26(1): 14–24.

Hu, W.; Fu, W.; Wei, X.; Yang, Y.; Lu, C. and Liu, Z. (2019). A Network Pharmacology Study on the Active Ingredients and Potential Targets of Tripterygium wilfordii Hook for Treatment of Rheumatoid Arthritis. Evidence-Based Complementary and Alternative Medicine, 2019, 1-15.

Jiang YS, Zhao S. (1987). Tripterygium wilfordii Hook induced acute toxicity with kidney involvement in 17 cases. Chin J Kidney Dis 3:167.

Klein, K.; Ospelt, C. and Gay, S. (2012). Epigenetic contributions in the development of rheumatoid arthritis. Arthritis Research & Therapy, 14(6): 227.

Lee, G.; Ha, J.; Min, K.; Nakagawa, H.; Tsurufuji, S.; Chang, I. and Kim, Y. (1995). Inhibitory Effects of Oriental Herbal Medicines on IL-8 Induction in Lipopolysaccharide-Activated Rat Macrophages. Planta Medica, 61(01): 26-30.

Li, X. and Weir, M. (1990). RADIX Tripterygium Wilfordii—a Chinese herbal medicine with potent immunosuppressive properties. Transplantation, 50(1): 82-85.

Liacini, A.; Sylvester, J. and Zafarullah, M. (2005). Triptolide suppresses proinflammatory cytokine-induced matrix metalloproteinase and aggrecanase-1 gene expression in chondrocytes. Biochemical and Biophysical Research Communications, 327(1): 320-327.

Lin, N.; Sato, T. and Ito, A. (2001). Triptolide, a novel diterpenoid triepoxide from Tripterygium wilfordii Hook. f.; suppresses the production and gene expression of pro-matrix metalloproteinases 1 and 3 and augments those of tissue inhibitors of metalloproteinases 1 and 2 in human synovial fibroblasts. Arthritis & Rheumatism, 44(9): 2193-2200.

Liu, L.; Zhao, H.; Sun, X.; Zheng, Q.; Luo, Y.; Ru, Y.; Zhang, Y.; Chen, X.; Zhu, B.; Yin, C.; Li, B. and Li, X. (2018). Efficacy and safety of Tripterygium wilfordii hook F for chronic urticaria: A systematic review and meta-analysis. BMC Complementary and Alternative Medicine, 18(1).

Liu, Q.; Chen, T.; Chen, G.; Li, N.; Wang, J.; Ma, P. and Cao, X. (2006). Immunosuppressant triptolide inhibits dendritic cell-mediated chemoattraction of neutrophils and T cells through inhibiting Stat3 phosphorylation and NF-κB activation. Biochemical And Biophysical Research Communications, 345(3): 1122-1130.

Luk, J.; Lai, W.; Tam, P. and Koo, M. (2000). Suppression of cytokine production and cell adhesion molecule expression in human monocytic cell line THP-1 by Tripterygium wilfordii polysaccharide moiety. Life Sciences, 67(2): 155-163.

Lv, Q. W.; Zhang, W.; Shi, Q.; Zheng, W. J.; Li, X.; Chen, H.; Wu, Q. J.; Jiang, W. L.; Li, H. Bin, Gong, L.; Wei, W.; Liu, H.; Liu, A. J.; Jin, H. T.; Wang, J. X.; Liu, X. M.; Li, Z. Bin, Liu, B.; Shen, M.; Kvien, T. K. (2015). Comparison of Tripterygium wilfordii Hook F with methotrexate in the treatment of active rheumatoid arthritis (TRIFRA): A randomised, controlled clinical trial. Annals of the Rheumatic Diseases, 74(6): 1078–1086.

Maekawa, K.; Yoshikawa, N.; Du, J.; Nishida, S.; Kitasato, H. and Okamoto, K. *et al*. (1999). The molecular mechanism of inhibition of interleukin-1β-induced cyclooxygenase-2 expression in human synovial cells by Tripterygium wilfordii Hook F extract. Inflammation Research, 48(11): 575-581.

Mao, S. and Huang, X. (2016). Tripterygium wilfordii Hook F is efficacious in the treatment of Henoch-Schönlein

purpura nephritis in children. World Journal ofPediatrics, 12(3): 375-376.

Qiu, D. and Kao, P. (2003). Immunosuppressive and Anti-Inflammatory Mechanisms of Triptolide, the Principal Active Diterpenoid from the Chinese Medicinal Herb Tripterygium wilfordii Hook. f. Drugs in R & D, 4(1): 1-18.

Qiu, D.; Zhao, G.; Aoki, Y.; Shi, L.; Uyei, A. and Nazarian, S. *et al*. (1999). Immunosuppressant PG490 (Triptolide) Inhibits T-cell Interleukin-2 Expression at the Level of Purine-box/Nuclear Factor of Activated T-cells and NF-κB Transcriptional Activation. Journal of Biological Chemistry, 274(19): 13443-13450.

Qiu, J.; You, X. and Wu, G. (2017). Effects of Tripterygium glycoside treatment on experimental autoimmune encephalomyelitis. Molecular Medicine Reports, 16, 8283-8288.

Scott, D. L.; Wolfe, F. and Huizinga, T. W. J. (2010). Rheumatoid arthritis. The Lancet, 376(9746): 1094–1108.

Singh, J.; Saag, K.; Bridges, S.; Akl, E.; Bannuru, R. and Sullivan, M. *et al*. (2015). 2015 American College of Rheumatology Guideline for the Treatment of Rheumatoid Arthritis. Arthritis & Rheumatology, 68(1): 1-26.

Si-Xun Yang, Shu-Sheng Xie, Han-Lin Gao, Da-Long Ma, & Zhen-Zhou Long. (1994). Triptolide suppresses T-lymphocyte proliferation by inhibiting interleukin-2 receptor expression, but spares interleukin-2 production and mRNA expression. International Journal Of Immunopharmacology, 16(11): 895-904.

Sylvester, J.; Liacini, A.; Li, W.; Dehnade, F. and Zafarullah, M. (2001). Tripterygium wilfordii Hook F Extract Suppresses Proinflammatory Cytokine-Induced Expression of Matrix Metalloproteinase Genes in Articular Chondrocytes by Inhibiting Activating Protein-1 and Nuclear Factor-κB Activities. Molecular Pharmacology, 59(5): 1196-1205.

Tao X, Cai JJ, Lipsky PE. (1995). The identity of immunosuppressive components of the ethyl acetate extract and chloroform methanol extract (T2) of Tripterygium wilfordii Hook. F. J Pharmacol Exp Ther 272:1305–1312.

Tao XL, Shi YP, Cheng XH. (1988). Mechanism of treating rheumatoid arthritis with Tripterygium wilfordii Hook. Effects on secretion of total IgM and IgM-rheumatoid factor by peripheral blood mononuclear cells. Zhongguo Yi XueKeXue Yuan Xue Bao 10:361–364. (in Chinese)

Tao, X. (1997). A Potential New Treatment for Rheumatoid Arthritis: Thunder God Vine. 5(5): 713–723.

Tao, X. L.; Sun, Y.; Dong, Y.; Xiao, Y. L.; Hu, D. W.; Shi, Y. P.; Zhu, Q. L.; Dai, H. and Zhang, N. Z. (1989). A prospective, controlled, double-blind, cross-over study of Tripterygium wilfodii hook F in treatment of rheumatoid arthritis. Chinese medical journal, 102(5): 327–332.

Tao, X. and Lipsky, P. E. (2000). The Chinese anti-inflammatory and immunosuppressive herbal remedy Tripterygium wilfordii Hook F. Rheumatic Disease Clinics of North America, 26(1): 29–50.

Tao, X.; Cush, J. J.; Garret, M. and Lipsky, P. E. (2001). A phase I study of ethyl acetate extract of the chinese antirheumatic herb Tripterygium wilfordii hook F in rheumatoid arthritis. The Journal of rheumatology, 28(10): 2160–2167.

Tao, X.; Davis, L. and Lipsky, P. (1991). Effect of an Extract of the Chinese Herbal RemedyTripterygiumWilfordii Hook F on Human Immune Responsiveness. Arthritis & Rheumatism, 34(10): 1274-1281.

Tao, X.; Ma, L.; Mao, Y. and Lipsky, P. (1999). Suppression of carrageenan-induced inflammation in vivo by an extract of the Chinese herbal remedy Tripterygium wilfordii Hook F. Inflammation Research, 48(3): 139-148.

Tao, X.; Schulze-Koops, H.; Ma, L.; Cai, J.; Mao, Y. and Lipsky, P. (1998). Effects of Tripterygium wilfordii Hook F extracts on induction of cyclooxygenase 2 activity and prostaglandin E2 production. Arthritis & Rheumatism, 41(1): 130-138.

Tao, X.; Younger, J.; Fan, F. Z.; Wang, B. and Lipsky, P. E. (2002). Benefit of an extract of Tripterygium wilfordii Hook F in patients with rheumatoid arthritis: A double-blind, placebo-controlled study. Arthritis and Rheumatism, 46(7): 1735–1743.

Tong, K.; Yang, D.; Chan, E.; Chiu, P.; Yau, K. and Lau, C. (1999). Downregulation of lymphocyte activity and human synovial fibroblast growth in rheumatoid arthritis by triptolide. Drug Development Research, 47(3): 144-153.

Wang, J.; Xu, R.; Jin, R.; Chen, Z. and Fidler, J. (2000). Immunosuppressive activity of the Chinese medicinal plant Tripterygium wilfordii. Transplantation, 70(3): 447-455.

Wang, X. andXie, H. (1999). Recent studies on Tripterygium wilfordii. Drugs of The Future, 24(9): 991.

Ye.WH, Tao.XL, Zhang NZ. (1990). Mechanism of treating rheumatoid arthritis with polyglycosides of Tripterygium wilfordii Hook. Zhongguo Yi XueKeXue Yuan Xue Bao 12:217–221. (in Chinese)

Yu, X.; Zhao, Q.; Zhang, X.; Zhang, H.; Liu, Y.; Wu, X.; Li, M.; Li, X.; Zhang, J.; Ruan, X. and Zhang, H. (2017). Celastrol ameliorates inflammation through inhibition of NLRP3 inflammasome activation. Oncotarget, 8(40): 67300–67314.

Zhang L, Zhang ZX, An DK. (1990). The achievements in the studies of chemical components of Tripterygium wilfordii Hook family. Zhongguo Yao Ke Da XueXue Bao 21(4): 251–256.

Zhang, Q.; Shi, Y.; Tan, W. and Wang, F. (2008). Effect of triptolide on the changes of VEGF and MMP-9 levels in the rheumatoid arthritis fibroblast-like synoviocyte line, MH7A. Nanjing Yi Ke Da XueXue Bao 28: 902–905. (in Chinese)

Zheng, J.R.; Fang, J.L.; Gu, K.X.; Ti, Y.Q.; Xu, L.F.; Gao, J.W. *et al*. (1987). Screening of active anti-inflammatory-immunosuppressive and antifertile components from *Tripterygium wilfordii*. II. Screening of 5 monomers from total glucosides of *Tripterygium wilfordii* (TII). Zhongguo Yi XueKeXue Yuan Xue Bao 9:323–328 (in Chinese)

Zheng, J.R.; Fang, J.L.; Gu, K.X.; Xu, L.F.; Gao, J.W.; Guo, H.Z. *et al*. (1987). Screening of active anti-inflammatory-immunosuppressive and antifertile compositions from *Tripterygium wilfordii*. I. Screening of 8 components from total glucosides of *Tripterygium wilfordii* (TII). Zhongguo Yi XueKeXue Yuan Xue Bao 9:317–322 (in Chinese)

Zhou, G.W.; Shen, Q.; Jin, R.J.; Wang, X.D.; Zeng, Y.X. and Ye, C.Y. (2007). Effects of Tripterygium glycosides (TG) on apoptosis of synovial fibroblasts in rheumatoid arthritis. Zhejiang Lin Chuang Yi Xue 9:1034–1035. (in Chinese)

Zhou, H.; Niu, D.; Xue, B.; Li, F.; Liu, X. and He, Q. *et al*. (2003). Triptolide inhibits TNF-α, IL-1β and NO production in primary microglial cultures. Neuroreport, 14(7): 1091-1095.