

DOCTORAL THESIS

The anti-arthritic effect and underlying mechanisms of QFGJS, a pharmaceutical preparation from a Chinese herbal formula

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**The Anti-arthritic Effect and Underlying Mechanisms
of QFGJS, a Pharmaceutical Preparation from a
Chinese Herbal Formula**

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A thesis submitted in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

Principal Supervisor: Prof. LIU Liang

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ABSTRACT

QFGJS is a pharmaceutical preparation from an anti-arthritis Chinese herbal formula composed of five well-documented herbs, and intended to be approved by the State Food and Drug Administration (SFDA) of China as a novel botanical drug for the treatment of rheumatoid arthritis (RA). The objective of this research is to comprehensively and intensively investigate the anti-arthritis effect and underlying mechanisms of QFGJS on experimental models of arthritis in rats including adjuvant-induced arthritis (AIA) and collagen-induced arthritis (CIA).

Through combination of chemical, pharmacodynamic, and toxicological studies, the optimized and standardized pharmaceutical procedures and manufacturing processes for the pilot production of QFGJS were established. Quality analysis of the pilot product of QFGJS by high-performance liquid chromatography (HPLC) demonstrated that the chromatographic fingerprint profiles of three batches of QFGJS were almost identical and the contents of four characteristic and bioactive markers were relatively consistent. General toxicological studies showed a favorable safety profile of QFGJS. The maximum tolerated single dose of QFGJS was determined in both sexes of rats to be 33.63 g/kg body weight which is equivalent to 346 times of clinical dose. In the chronic oral toxicity study, significant loss of body weight of animals treated with doses of 3.89, 6.80, and 9.72 g/kg body weight (equivalent to 40, 70 and 100-fold clinical doses, respectively) was observed after 6 weeks of daily administration of QFGJS. However, the results of laboratory investigation showed that QFGJS caused no changes in all hematological parameters and blood biochemical parameters of rats. No mortality or specific toxic responses were observed in animals after 3 months of repeated dosing with QFGJS.

To establish a well-developed and well-characterized AIA model in the outbred Sprague-Dawley (SD) rats for the evaluation of anti-arthritis effect of QFGJS, the roles of different preparative techniques, inoculation routes and doses of *Mycobacterium tuberculosis* (MT) suspension as well as the sex preference in the induction of AIA in SD rats were comparatively studied using various examinations. The results demonstrated that the particle size and dose of MT in the suspension played a dominant role in the induction and severity of AIA. The same incidence and no significantly different severity of AIA were observed in the rats inoculated either intradermally or subcutaneously. Male rats manifested markedly more severe arthritic signs than female rats. After subcutaneous inoculation with the ground MT suspension containing 500 μg MT, male SD rats developed pronounced arthritis with 100% incidence and low variable clinical signs. Even only 62.5 μg MT was used for inoculation, AIA was efficiently induced in male rats, which showed up-regulated expression of interleukin (IL)-1 β , IL-6, and tumor necrosis factor (TNF)- α . Moreover, we compared AIA in SD and Lewis rats in terms of clinical, histological, radiological, and immunoinflammatory features. The results showed that, following inoculation with the ground MT suspension, both strains of rats experienced closely similar disease progression, with 100% incidence, similar severity and low variability in clinical arthritic signs. The development of arthritis was accompanied by significantly higher erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels than in control rats. Radiographic examination of the hind paws showed that both SD and Lewis AIA rats manifested conspicuous soft tissue swelling, bone matrix resorption, periosteal new bone formation, and bone erosion, while histopathological analysis of the synovial

joints revealed marked cellular infiltration, angiogenesis, synovial hyperplasia, pannus formation, narrowing of joint space, and focal erosions of cartilage and bone. Moreover, in relation to disease progression, serum TNF- α , IL-1 β , and IL-6 levels were markedly overproduced in both SD and Lewis AIA versus control rats, and SD and Lewis AIA rats exhibited divergent profiles for the expression of TNF- α and IL-1 β . Taken together, these results demonstrated that the SD rat AIA model shares several arthritic features with the comparable model in Lewis rats. Hence, given the more favorable characteristics of SD rats as compared with Lewis rats, i.e., lower cost, wider availability, and heterogenic background, this SD rat AIA model is much more cost effective and advantageous for studying the pathophysiology of arthritis in humans as well as for screening novel anti-arthritic agents. This is also one of the findings and contributions to the academic community in the current research.

To analyze the anti-arthritic effect of QFGJS on RA, the well-established AIA model in SD rats and CIA model in Wistar rats were used in the current study. Two treatment protocols, i.e., oral administration with different doses of QFGJS (0.97, 1.94, and 3.89 g/kg body weight), or indomethacin at the dose of 1 mg/kg body weight, or vehicle beginning on the day of the induction of arthritis (the prophylactic treatment protocol) or on the day after the onset of arthritis (the therapeutic treatment protocol), were initiated and continued until day 30 of the experiments. The results showed that prophylactic treatment with QFGJS significantly suppressed the onset of arthritis, while therapeutic treatment with QFGJS markedly reduced arthritic score, paw swelling, and ESR levels even in the established arthritis. Radiological and histopathological examinations showed markedly decreased tissue and bone destruction of arthritic joints in the QFGJS-treated rats. The current study demonstrates that oral treatment with QFGJS can effectively block the disease progression of arthritis, showing suppression of joint inflammation and of the radiological and histopathological progression of joint damage.

The possible molecular mechanisms underlying the anti-arthritic effect of QFGJS in which pro-inflammatory cytokines TNF- α , IL-1 β , and IL-6 are predominantly involved were further investigated. Our results showed that the inflammatory process in the arthritic rats led to substantial increases in systemic levels of TNF- α , IL-1 β , and IL-6; while QFGJS decreased overexpressed TNF- α , IL-1 β , and IL-6 levels in blood serum in a dose-dependent manner. Therefore, the anti-arthritic effect of QFGJS on both polyarticular inflammation and joint damage may basically correlate with its action of suppressing the abundant production of TNF- α , IL-1 β , and IL-6 in blood serum.

Overall, QFGJS has been demonstrated to not only direct towards the control of pain and the inflammation associated with joint synovitis, but also prevent the structural damage of arthritic joints caused by tissue and bone breakdown. QFGJS is an example of combination therapy of Chinese medicinal herbs, which, increasingly, is being found to be the best approach to complex refractory and degenerative diseases. The results obtained in our studies provide substantial and well-documented evidence of the quality, safety, and effectiveness of QFGJS as an anti-arthritic agent. Hence, QFGJS is a strong candidate for development into an effective botanical drug for the treatment of RA.

TABLE OF CONTENTS

DECLARATION.....	i
ABSTRACT.....	ii
ACKNOWLEDGEMENTS.....	iv
TABLE OF CONTENTS.....	vi
LIST OF TABLES.....	xiii
LIST OF FIGURES.....	xiv
LIST OF ABBREVIATIONS.....	xvii
PART I INTRODUCTION AND DESIGN OF THE RESEACH.....	1
1.1 Pathogenesis of Rheumatoid Arthritis.....	1
1.2 Therapeutic Approaches of Rheumatoid Arthritis.....	5
1.3 Therapeutic Intervention of Rheumatoid Arthritis with Chinese Medicine.....	8
1.4 QFGJS and Its Potential as a Novel Anti-arthritic Botanical Drug Product.....	10
1.5 Experimental Models of Human Arthritis.....	14
1.5.1 Adjuvant-induced Arthritis (AIA).....	14
1.5.2 Pristane-induced Arthritis (PIA).....	16
1.5.3 Streptococcal Cell Wall-induced Arthritis (SCWA).....	18
1.5.4 Collagen-induced Arthritis (CIA).....	20
1.5.5 Proteoglycan-induced Arthritis (PGIA).....	22
1.6 Aims and Design of the Research.....	23
1.6.1 Aims and Objectives of the Research.....	23
1.6.2 Design of the Research.....	24
1.6.3 Structure of the Thesis.....	27

PART II SCREENING FOR THE OPTIMAL PHARMACEUTICAL PROCESS OF QFGJS PRODUCTION AND QUALITY AND SAFETY ASSESSMENT OF QFGJS PILOT PRODUCT.....	28
1. Introduction.....	28
2. Materials and Methods.....	30
2.1 Sources and Authentication of Herbs.....	30
2.2 Preparation of Different Pharmaceutical Extracts of the QFGJS Formula.....	31
2.3 Pilot Manufacturing of QFGJS.....	33
2.4 Chemicals and Reagents.....	34
2.5 Determination of Representative Bioactive Chemicals in Different Extracts of the QFGJS Formula using HPLC Method.....	34
2.6 Quality Assessment of the Pilot Product of QFGJS by HPLC Fingerprinting.....	35
2.7 Experimental Animals.....	36
2.8 Anti-inflammatory Test of Different Extracts of the QFGJS Formula.....	36
2.9 Antinociceptive Tests of Different Extracts of the QFGJS Formula.....	37
2.10 Acute Toxicity Test of Different Extracts of the QFGJS Formula	38
2.11 General Toxicity Study of the Pilot Product of QFGJS	38
2.12 Statistical Analysis.....	39
3. Results.....	39
3.1 Quantitative Determination of Representative Bioactive Chemicals in Different Extracts of the QFGJS Formula.....	40
3.2 Anti-inflammatory Effect of Different Extracts of the QFGJS Formula.....	40
3.3 Antinociceptive Effect of Different Extracts of the QFGJS Formula.....	41
3.4 Acute Toxicity of Different Extracts of the QFGJS Formula.....	43

3.5 Characteristics of the HPLC Fingerprints of the Pilot Product of QFGJS.....	44
3.6 General Toxicity of the Pilot Product of QFGJS.....	44
4. Discussion.....	50
PART III DEVELOPMENT AND CHARACTERIZATION OF ADJUVANT- INDUCED ARTHRITIS IN SPRAGUE-DAWLEY RATS.....	
Chapter 1 Manipulation of the Induction of Adjuvant Arthritis in Sprague-Dawley Rats.....	54
1.1 Introduction.....	54
1.2 Materials and Methods.....	56
1.2.1 Preparation of Mycobacteria Suspension.....	57
1.2.2 Induction of AIA.....	57
1.2.3 Clinical Assessment of AIA.....	58
1.2.4 Haematological Examination and Measurement of Serum Cytokines Levels.....	58
1.2.5 Radiological and Histological Examinations.....	59
1.2.6 Statistical Analysis.....	60
1.3 Results.....	60
1.3.1 Susceptibility of SD and Lewis Rats to AIA.....	60
1.3.2 Difference in Arthritogenic Ability of the Commercial CFA and Ground MT Suspension.....	63
1.3.3 Difference in Severity of AIA Induced by Different Inoculation Routes.....	63
1.3.4 Sex Preference in Incidence and Severity of AIA.....	68
1.3.5 Influence of the Doses of MT in the Suspension on Incidence and Severity of AIA.....	68

1.3.6 Influence of MT Particle Size on Arthritogenic Ability of the MT Suspension.....	68
1.3.7 Correlations of Clinical Arthritic Signs and the Levels of ESR and Serum Cytokines in AIA Rats.....	77
1.3.8 Radiological Examination of Hind Paws of AIA and Control Rats.....	82
1.3.9 Histological Examination of Ankle Joints of AIA and Control Rats.....	82
1.4 Discussion.....	87
Chapter 2 The Comparative Study of Sprague-Dawley and Lewis Rats in Adjuvant- induced Arthritis.....	94
2.1 Introduction.....	94
2.2 Materials and Methods.....	96
2.2.1 Preparation of Mycobacteria Suspension.....	96
2.2.2 Induction of AIA.....	96
2.2.3 Clinical Evaluation of the Development of AIA.....	97
2.2.4 Determination of ESR, CRP and Pro-inflammatory Cytokines Levels.....	97
2.2.5 Radiological and Histopathological Studies.....	98
2.2.6 Statistical Analysis.....	99
2.3 Results.....	99
2.3.1 Clinical Progression of AIA in SD and LEW Rats.....	99
2.3.2 Radiological Analysis of AIA in SD and LEW Rats.....	104
2.3.3 Histopathological Analysis of AIA in SD and LEW Rats.....	104
2.3.4 Expression Profiles of Serum Cytokines Levels of AIA in SD and LEW Rats.....	109
2.4 Discussion.....	112

PART IV THE ANTI-ARTHRITIC EFFECT AND UNDERLYING MECHANISMS OF QFGJS.....	117
Chapter 1 Suppressive Effects of QFGJS on Rat Experimental Adjuvant-induced Arthritis.....	118
1.1 Introduction.....	118
1.2 Materials and Methods.....	120
1.2.1 Sources and Authentication of Herbs.....	120
1.2.2 Preparation of QFGJS.....	121
1.2.3 Animals.....	121
1.2.4 Induction of AIA and QFGJS Treatment.....	122
1.2.5 Assessments of the Arthritis Severity and the Effects of the Treatments.....	122
1.2.6 Measurements of ESR and Serum Cytokines Levels.....	123
1.2.7 Radiological and Histopathological Examinations.....	123
1.2.8 Statistical Analysis.....	124
1.3 Results.....	125
1.3.1 Inhibitory Effects of QFGJS Administered From the Day of Arthritis Induction.....	125
1.3.2 Therapeutic Effects of QFGJS Administered From the Day of Arthritis Onset.....	125
1.3.3 Protective Effects of QFGJS on Cartilage and Bone Destruction.....	131
1.3.4 Effects of QFGJS on Serum TNF- α , IL-1 β and IL-6 Levels.....	131
1.4 Discussion.....	136
Chapter 2 Suppression of the Onset and Progression of Collagen-induced Arthritis in Rats by QFGJS.....	142
2.1 Introduction.....	142

2.2 Materials and Methods.....	144
2.2.1 Preparation of QFGJS.....	144
2.2.2 Animals.....	145
2.2.3 Induction of CIA and QFGJS Treatment.....	145
2.2.4 Evaluation of the Development of Arthritis.....	146
2.2.5 Measurements of ESR and Serum Cytokines Levels.....	146
2.2.6 Radiological and Histopathological Examinations.....	147
2.2.7 Statistical Analysis.....	148
2.3 Results.....	148
2.3.1 Effects of Prophylactic Treatment with QFGJS on the Onset and Disease Progression of CIA.....	148
2.3.2 Effects of Therapeutic Treatment with QFGJS on the Disease Progression of the Established CIA.....	149
2.3.3 Effects of QFGJS on Radiological Changes of Joint Damage of the CIA Rats.....	155
2.3.4 Effects of QFGJS on Histological Changes of Joint Damage of the CIA Rats.....	155
2.3.5 Effects of QFGJS on Serum TNF- α , IL-1 β and IL-6 Levels.....	160
2.4 Discussion.....	160
Chapter 3 The Anti-arthritic Effect of Sinomenine on Collagen-induced Arthritis in Rats.....	166
3.1 Introduction.....	166
3.2 Materials and Methods.....	170
3.2.1 Drugs and Reagents.....	170
3.2.2 Animals.....	171

3.2.3 Induction of CIA.....	171
3.2.4 Treatment.....	172
3.2.5 Evaluation of the Development of Arthritis.....	172
3.2.6 Measurement of ESR.....	172
3.2.7 Statistical Analysis.....	173
3.3 Results.....	173
3.3.1 Influence of Sinomenine on Incidence of CIA.....	173
3.3.2 Effect of Sinomenine on Disease Progression of CIA.....	175
3.3.3 Effect of Sinomenine on ESR of CIA.....	175
3.4 Discussion.....	179
PART V SUMMARY AND PROSPECTIVE OF THE RESEARCH.....	181
1. Summary and Conclusions of the Studies.....	181
2. Prospective for Further Studies.....	190
REFERENCES.....	194
PUBLICATIONS.....	224
PRESENTATIONS AND ABSTRACTS.....	226
CURRICULUM VITAE.....	227