ABSTRACT

Parkinson’s disease (PD) is the second most common neurodegenerative disease affecting 2% of the population over 65 years old that lacks effective cure. The current available treatments for PD are largely symptomatic and palliative. Tianma Gouteng Yin (TGY) is a traditional Chinese medicine (TCM) formula belongs to the formulas that dispel wind. Nowadays, it has been a commonly prescribed formula to treat Parkinsonian-like symptoms such as tremor and paralysis in some of the patients. However, just as most of the TCM formula, the material basis and the underlying pharmacological effects of TGY are still lacking experimental evidence.

In this study, a method using UHPLC/Q-TOF-MS and HPLC-ELSD has been developed and successfully applied to qualitatively and quantitatively determine the complex phytochemicals of TGY. Totally 28 phytochemicals were identified, of which 20 were simultaneously quantified. The material basis profile of TGY decoction was delineated for the first time.

After full component analysis of TGY, the neuroprotective activity of TGY was verified both in vivo and in vitro. In Drosophila PD models, TGY mitigated rotenone induced toxicity and promoted α-synuclein clearance. In stereotaxic rotenone intoxication rats, TGY exerted neuroprotective effects in terms of preventing dopaminergic neurons loss and alleviating neuroinflammation. TGY alleviated rotenone induced apoptosis in SH-SY5Y cells. Furthermore, we discovered that Geniposide, an important component of TGY, is an autophagy inducer both in vivo and in vitro and is neuroprotective in
transgenic *Drosophila* PD model. In general, our study proves that TGY is neuroprotective in PD models.

In addition to the efficacy study, safety of TGY application in terms of TGY-drug interaction was also evaluated. In our study, herb-drug interactions between TGY and one of the most popular drugs used in PD treatment, Sinemet, were studied. The pharmacokinetics data showed that co-administration of TGY could suppress the absorption of Levodopa, the main component of Sinemet, for the first time. This information suggest that in clinical practice, TGY should avoid been administrated with Levodopa containing medicaments at the same time.

In conclusion, the data of this study provides valuable information on the material basis, efficacy and safety of TGY. This information is useful reference for the clinical application of TGY in PD treatment.

**Keywords:** Tianma Gouteng Yin, Parkinson’s disease, *Drosophila*, Rotenone, α-synuclein, Geniposide, Autophagy, Sinemet, Herb-drug interaction
TABLE OF CONTENTS

DECLARATION ...........................................................................................................I

ABSTRACT ................................................................................................................II

ACKNOWLEDGEMENTS ............................................................................................IV

TABLE OF CONTENTS ...........................................................................................V

LIST OF TABLES .....................................................................................................XII

LIST OF ABBREVIATION ........................................................................................XIII

CHAPTER 1. INTRODUCTION ................................................................................1

CHAPTER 2. OBJECTIVES OF THE STUDY .........................................................20

CHAPTER 3. COMPONENTS ANALYSIS OF TGY ...............................................22

  3.1 INTRODUCTION ..............................................................................................22

  3.2 MATERIALS AND METHODS .........................................................................24

    3.2.1 Reagents, chemicals and other materials ..................................................24

    3.2.2 Preparation of TGY extract ......................................................................25

    3.2.3 Sample solutions preparation ...................................................................25

    3.2.4 Standard solutions preparation ...............................................................26

    3.2.5 Analytical method ....................................................................................27

    3.2.6 Method validation ....................................................................................28

  3.3 RESULTS ..........................................................................................................29

    3.3.1 Preparation of TGY water extract ............................................................29

    3.3.2 Optimization of the chromatographic conditions .....................................31
3.3.3 Identification of chemical compounds .......................................................... 33

3.3.4 Method validation ............................................................................................. 45

3.3.5 Quantification of compounds in TGY .............................................................. 47

3.4 CONCLUSION AND DISCUSSION ...................................................................... 49

CHAPTER 4. TGY IS NEUROPROTECTIVE IN PD MODELS .............................. 51

4.1 INTRODUCTION ................................................................................................. 51

4.2 MATERIALS AND METHODS .......................................................................... 52

4.2.1 Reagents and antibodies .................................................................................. 52

4.2.2 Preparation of drug-containing fly food .......................................................... 53

4.2.3 Drosophila culture and strains ......................................................................... 53

4.2.4 Survival assay ................................................................................................... 53

4.2.5 Climbing assay .................................................................................................. 54

4.2.6 Immunohistochemical analysis of Drosophila samples ..................................... 54

4.2.7 Immunoblotting analysis of Drosophila samples ............................................. 55

4.2.8 Housing and husbandry of rats ....................................................................... 55

4.2.9 Stereotaxic rotenone intoxication PD model and TGY treatment ..................... 56

4.2.10 Apomorphine (APO)-induced rotation .......................................................... 57

4.2.11 Immunostaining of rat brain samples ............................................................. 57

4.2.12 Immunoblot analysis of rat brain samples ...................................................... 58

4.2.13 Dopamine quantitation .................................................................................... 59

4.2.14 Cell culture and cell viability assay ............................................................... 60

4.2.15 Statistics analysis ............................................................................................ 61

4.3 RESULTS ........................................................................................................... 61

4.3.1 TGY antagonized rotenone toxicity in Drosophila ........................................... 61
4.3.2 TGY reduced α-synuclein level and suppressed neurotoxicity in transgenic Drosophila ............................................................... 63
4.3.3 TGY reduced α-synuclein induced dopaminergic neuron loss in transgenic Drosophila ............................................................. 65
4.3.4 TGY attenuated APO-provoked rotational behavior in rotenone-intoxicated PD rats ........................................................................ 67
4.3.5 TGY alleviated dopaminergic neuron loss induced by rotenone intoxication .......................................................... 68
4.3.6 TGY relieved neuroinflammation in rotenone-intoxicated PD rats ........... 73
4.3.7 TGY reduced rotenone-induced apoptotic cell death in SH-SY5Y cells .. 76

4.4 DISCUSSION AND CONCLUSION ......................................................................................................................... 77

CHAPTER 5. GENIPOSIDE, A PRINCIPAL COMPONENT OF TGY, PROMOTES AUTOPHAGY BOTH IN VIVO AND IN VITRO .......................................................... 80

5.1 INTRODUCTION ................................................................................................................................. 80

5.2 MATERIALS AND METHODS ............................................................................................................. 82

5.2.1 Reagents and antibodies ................................................................................................................. 82
5.2.2 Cell lines and cell culture .............................................................................................................. 82
5.2.3 Protein samples preparation and immunoblot analysis .............................................................. 82
5.2.4 Fluorescence analysis of cell puncta ............................................................................................. 83
5.2.5 Primary neuron culture ................................................................................................................. 83
5.2.6 Drosophila strains and culture ..................................................................................................... 85
5.2.7 Confocal analysis of Atg8a-mCherry-EGFP puncta in Drosophila fat body .................................... 85

5.3 RESULTS .................................................................................................................................................. 86

5.3.1 Geniposide upregulated LC3II level in neuronal cell lines ...................................................... 86
5.3.2 Geniposide increased LC3II level in primary rat cortical neurons .......... 88
5.3.3 Geniposide promoted autophagic flux in N2a cell. ............................... 91
5.3.4 Geniposide enhanced autophagic flux in Drosophila ......................... 93
5.3.5 Geniposide reduced α-synuclein level and suppressed neurotoxicity in transgenic Drosophila ................................................................. 95
5.3.6 Geniposide induced autophagy in neuronal cells in an mTOR-independent manner ................................................................. 97
5.4 CONCLUSION AND DISCUSSION ....................................................... 98

CHAPTER 6. PHARMACOKINETICS INTERACTIONS BETWEEN TGY AND SINEMET .................................................................................. 101
6.1 INTRODUCTION .............................................................................. 101
6.2 MATERIALS AND METHODS ............................................................... 103
6.2.1 Materials and reagents .................................................................. 103
6.2.2 Preparation of standards solutions and quality control (QC) samples .... 103
6.2.3 Sample preparation ....................................................................... 104
6.2.4 Analytical method ......................................................................... 104
6.2.5 Method validation ........................................................................... 105
6.2.6 Pharmacokinetics interaction of TGY and Sinemet in rats ............... 106
6.3 RESULTS .......................................................................................... 107
6.3.1 Method validation ........................................................................... 107
6.3.2 Pharmacokinetics interaction between TGY and Sinemet ............... 113
6.4 CONCLUSION AND DISCUSSION ....................................................... 116

CHAPTER 7. GENERAL DISCUSSION AND CONCLUSION ...................... 118
7.1 MATERIAL BASIS OF TCM FORMULA ........................................ 118
7.2 TGY IN PD TREATMENT ................................................................. 119