Neuroprotective effect of
*Coptis chinensis*

**Dissertation**
with the aim of achieving a doctoral degree
at the Faculty of Mathematics, Informatics and Natural Sciences
Department of Biology
of Universität Hamburg

submitted by
Thomas Friedemann
from Gotha

Hamburg 2015
Contents

1. Summary 1

2. Introduction 7
   2.1. Oxidative stress and neurodegeneration .......................... 7
       2.1.1. Reactive Oxygen Species and oxidative stress ............. 8
       2.1.2. Cellular antioxidants defence system and oxidative damage ... 13
       2.1.3. Oxidative damage to proteins ............................... 14
       2.1.4. Oxidative damage to DNA .................................. 15
       2.1.5. Oxidative damage to lipids ................................ 15
       2.1.6. Repair and replacement .................................... 16
       2.1.7. Oxidative stress induced apoptosis .......................... 17
   2.2. Oxidative stress associated neurodegenerative disease ........ 19
       2.2.1. Parkinson's disease ....................................... 20
       2.2.2. Mitochondrial dysfunction and oxidative stress in PD .... 21
       2.2.3. Cellular – and animal models of PD .......................... 23
       2.2.4. Screening of Chinese herbs for promoting neuroprotection ... 23
   2.3. Aim of this thesis ................................................. 26

3. Coptis chinensis (Franch.) exhibits neuroprotective properties against oxidative stress in human neuroblastoma cells 27
   3.1. Introduction ...................................................... 29
   3.2. Materials and Methods ........................................... 30
       3.2.1. Drugs ...................................................... 30
       3.2.2. Herbal preparation ....................................... 31
       3.2.3. HPLC-Analysis ............................................. 31
       3.2.4. Free radical scavenging activity ............................ 31
       3.2.5. Peroxyl radical scavenging activity ........................ 32
       3.2.6. Cell culture .............................................. 32
       3.2.7. Cell viability measurements ................................ 32
       3.2.8. Quantification of ROS ..................................... 33
5. Neuroprotective effect of *Coptis chinensis* (Franch.) in MPP\(^+\) and MPTP induced Parkinson's disease models. 65

5.1. Introduction 66

5.2. Materials and Methods 68

5.2.1. Drugs and reagents 68

5.2.2. Herbal preparation 68

5.2.3. HPLC-Analysis 68

5.2.4. Cell culture 68

5.2.5. Effect of CRE and its main alkaloids on cell viability 69

5.2.6. Effect of CRE and its main alkaloids on ATP level 69

5.2.7. Effect of CRE on MPP\(^+\) induced apoptosis 69

5.2.8. Parkinson disease mouse model and pharmacological treatment 70

5.2.9. Rotarod test 70

5.2.10. Pole test 71

5.2.11. Brain tissue preparation 71

5.2.12. Stereology 72

5.2.13. Statistics 72

5.3. Results 72

5.3.1. HPLC-Analysis 72

5.3.2. Effect of CRE on cell viability 72

5.3.3. Effect of CRE on ATP concentration 73

5.3.4. Effect of CRE on apoptotic rate 74

5.3.5. Effect of CRE on Rotarod test 76

5.3.6. Effect of CRE on Pole-Test 76

5.3.7. Effect of CRE on TH+ Cells in SN and VTA 77

5.4. Discussion 77

5.5. Conclusion 81

5.6. Acknowledgements 81

6. General Discussion 83

6.1. Extraction and analysis of medical plants 83

6.2. Screening Chinese medical plants for neuroprotective effects against oxidative stress induced cytotoxicity 84

6.3. *Coptis chinensis* (Franch.) 87

6.4. Selection of single compounds of the CRE 88
6.5. Neuroprotective effect of *Coptis chinensis* and its main alkaloids against oxidative stress induced cytotoxicity ............................................ 89
6.5.1. Cell free assays ........................................... 89
6.5.2. Cell viability ........................................... 91
6.5.3. ROS .................................................. 93
6.5.4. Mitochondrial membrane potential ......................... 93
6.5.5. Apoptosis .............................................. 94
6.5.6. Transcriptional changes .................................. 96
6.5.7. Impact for the treatment of neurodegenerative disease associated with oxidative stress ......................................................... 98
6.6. Neuroprotective effect of *Coptis chinensis* in the MPP⁺ and MPTP induced Parkinson’s disease models ........................................ 99
6.6.1. Neuroprotective effect of *Coptis chinensis* and its main alkaloids in MPP⁺ induced in-vitro Parkinson’s disease models ......... 101
6.6.2. Neuroprotective effect of *Coptis chinensis* and its main alkaloids in MPTP induced in-vivo Parkinson’s disease models .......... 103
6.7. Future Perspective ........................................ 105

References ......................................................... 107

A. Appendix ......................................................... 131
A.1. Hypothesis driven screening of Chinese herbs for promoting neuroprotection ................................................................. 131
A.1.1. Lack of neuroprotective medicines ........................ 132
A.1.2. The screening modalities are the bottleneck ............... 132
A.1.3. Hypothesis driven screening ................................ 133
A.1.4. Finding herbs for PD ................................... 135
A.1.5. Acknowledgements ....................................... 136
A.1.6. References ............................................. 136
A.2. Publications, presentations, patent and awards ............... 139
A.2.1. Publications integrated in this thesis .................... 139
A.2.2. Publications not integrated in this thesis .................. 140
A.2.3. Oral presentations ..................................... 141
A.2.4. Poster ................................................. 142
A.2.5. Patent .................................................. 144
A.2.6. Awards ................................................. 144
A.3. Record of contribution .................................... 147
A.4. Original publications ..................................... 149