

**Pharmacological characterization of new neuroprotectants
in Parkinson's disease models**

by

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Doctor of Philosophy in Biomedical Sciences



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Abstract

Parkinson's disease (PD) ranks second in the prevalence of neurodegenerative disorders among the elderly. Current therapies to PD mainly focus on relieving symptoms, and there is no effective drug to prevent, halt or slow the progression of the disease. Earlier studies highlight the multi-pathological factors involved in PD. It is thus interesting to investigate the potential treatment of PD with the multi-target approach of Traditional Chinese medicine (TCM) that bases on a holistic and integrated theory for disease treatment.

TCMs particularly ameliorate the ageing-related symptoms, and hence are likely to be beneficial for chronic diseases such as PD. Chinese medicinal herbs which contain multiple chemical components are rich sources of bioactive compounds for the multi-target strategy in improving the treatment of PD. In an attempt to identify effective neuroprotectants for the prevention/treatment of PD, a panel of herbal extracts and a list of pure compounds with anti-inflammation, anti-oxidant, anti-ageing and tonifying activities were selected for screening in neurotoxin-induced PD model. Data from preliminary screening showed that the ethanolic extract of *Fructus Alpiniae oxyphyllae* (AOE) and pure compounds including quercetin and SU4312 exhibited promising neuroprotective effects. They were then systematically evaluated in established PD experimental cellular and zebrafish models, and the mechanisms underlying the neuroprotective activities were addressed. Major achievements of this study are summarized as follows:

(1) AOE was demonstrated for the first time to prevent and restore 6-OHDA-induced DA neuron loss in zebrafish. Pre-incubation with AOE increased

the viability of 6-OHDA-treated PC12 cells *in vitro* by attenuating cellular apoptosis. The mechanism underlying its neuroprotective effect involved anti-inflammation, anti-oxidant and activation of neuronal survival related phosphatidylinositol 3-kinase (PI3K)/Akt pathway.

(2) Two polyphenols present in AOE, namely PCA and chrysin, exhibited enhanced protective effect against 6-OHDA-induced damage in PC12 cells *in vitro* and DA neuron in zebrafish through activation of Nrf2/ARE signaling pathway. These two compounds might partially contribute to the neuroprotective action of AOE.

(3) The present study showed that quercetin could prevent (< 3 day post fertilization (dpf) zebrafish embryos) but not rescue (> 3 dpf zebrafish embryos) the zebrafish DA neuron loss induced by 6-OHDA when quercetin was administered at different maturation stages of the BBB in zebrafish. This provides indirect evidence of the presence of a functional BBB at 3dpf zebrafish larvae and an important role of BBB permeability in determining the beneficial effect of quercetin in PD *in vivo*.

(4) SU4312, a potent and selective inhibitor of vascular endothelial growth factor receptor-2, for the first time, was demonstrated to exhibit significantly neuroprotective effect against MPP⁺-induced damage of rat cerebella granule neurons, and ameliorated the loss of DA neurons and the impairment of swimming behavior of zebrafish caused by MPTP. The neuroprotective effect of SU4312 did not correlate with its anti-angiogenic activities, but *via* off-target inhibition of neuronal nitric oxide synthase.

In summary, the present study provided scientific rationale for the use of *Alpinia Oxyphyllae* and its active ingredients, as well as SU4312, in the prevention/treatment of PD and may lead to development of potential preventive agents for PD.

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List of Abbreviations

6-OHDA	6-hydroxydopamine
AD	Alzheimer's disease
Akt	Anti-apoptotic kinase
ALS	Amyotrophic lateral sclerosis
AO	Acridine orange
AOE	Ethanol extract of <i>Fructus Alpiniae oxyphyllae</i>
ARE	Antioxidant response element
BBB	Blood brain barrier
CGNs	Cerebellum granular neurons
ChE	Cholinesterase
CNS	Central nervous system
COMT	Catechol-O-methyl transferase
CoQ10	Coenzyme Q10
COX	Cyclooxygenase
DA	Dopaminergic
DAF-FM	4-amino-5-methylamino-2', 7'- difluorescein
DAPI	4'-6-Diamidino-2-phenylindole
DAT	Dopamine transporters
DMSO	Dimethyl sulfoxide
dpf	Days post fertilization
EGCG	(-)-epigallocatechin-3-gallate
eNOS	Endothelial nitric oxide synthase
ERK1/2	Extracellular-signal-regulated kinases 1/2
F-12K	Kaighn's modification of Ham's F12 medium
FBS	Fetal bovine serum
HO-1	Heme oxygenase-1
IL-1 β	Interleukin 1 beta
iNOS	Inducible nitric oxide synthetase
JNK	c-Jun N-terminal kinase
LDH	Lactate dehydrogenase
L-dopa	Levodopamine
LGA	L-hydroxyglutaric acid
L-NAME	N ω -nitro-L-arginine methyl ester hydrochloride
MAO-B	Monoamine oxidase-B
MDA	Malondialdehyde
MPP ⁺	1-methyl-4-phenylpyridinium ion
MPTP	1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine
MTT	3-[4, 5-dimethyl- thiazol-2-yl]-2, 5-diphenyl tetrazolium bromide

ND	Neurodegenerative diseases
nNOS	Neuronal NOS
NO	Nitric oxide
NOS	Nitric oxide synthase
Nrf2	Nuclear factor erythroid 2-related factor 2
PBS	Phosphate-buffered saline
PBST	Phosphate Buffered Saline Tween-20
PCA	Protocatechuic acid
PD	Parkinson's disease
PI3K	Phosphatidylinositol 3-kinase
PINK	Phosphatase and tensin homolog (PTEN)-induced putative kinase
PMSF	Phenylmethylsulfonyl fluoride
PTEN	Phosphatase and tensin homolog
ROS	Reactive oxygen species
RT-PCR	Reverse transcription PCR
siRNA	Short interfering ribonucleic acid
SNpc	Substantia nigra pars compacta
SOD	Superoxide dismutase
SU4312	3-[4-(dimethylamino)benzylidenyl]indolin-2-one
TCMs	Traditional Chinese medicines
TH	Tyrosine hydroxylase
TNF- α	Tumor necrosis factor alpha
VEGF	Vascular endothelial growth factor
VEGFR-2	Vascular endothelial growth factor receptor-2
vmat2	Vesicular monoamine transporter 2