Contents

1 Introduction 1

1.1 Epidemiology 1

1.2 Causes of cerebral stroke 2

1.3 Ischemic stroke 3

1.4 Clinical diagnosis of stroke 3

1.5 Animal models of focal ischemia 3

1.6 Platelet contribution to ischemic stroke 4

1.7 Treatment and prevention of stroke 5

1.8 Limitations of current treatments of stroke 6

1.9 Mitochondrial dysfunction in ischemic stroke 8

1.10 Mitochondrial Biogenesis 9

1.11 Indole-3-carbinol 10

1.12 Diindolylmethane 11

1.13 Rationale 11

1.14 Objectives 15

2 Introduction 17

2.1 Materials and methods 18

2.1.1 Chemicals 18

2.1.2 Experimental animals and drug treatment 18

2.1.3 Preparation of standard solutions 21

2.1.4 Preparation of calibration and quality control samples 21

2.1.5 Extraction of I3C and DIM 21

2.1.6 HPLC system 22

2.1.7 Pharmacokinetics 22
2.1.8 Middle cerebral artery occlusion and reperfusion injury 22
2.1.9 Neurological deficits 23
2.1.10 Brain infarction 23
2.1.11 Evaluation of blood brain barrier integrity 24
2.1.12 Brain water content 24
2.1.13 Data Analysis 25
2.2 Results 25
2.2.1 Linearity and sensitivity 25
2.2.2 Precision and accuracy 25
2.2.3 Stability studies 28
2.2.4 Recovery 30
2.2.5 Plasma concentration of I3C and DIM 30
2.2.6 Pharmacokinetic parameters 33
2.2.7 Tissue distribution 35
2.2.8 Brain concentrations of I3C and DIM 35
2.2.9 I3C and DIM concentrations in CSF 38
2.2.10 I3C alleviated the neurological deficits 40
2.2.11 I3C ameliorated the brain infarction 41
2.2.12 I3C mitigated the BBB leakage 42
2.2.13 I3C alleviated the brain edema 44
2.3 Discussion 45
2.4 Summary 49
3 Introduction 52
3.1 Materials and Methods 54

3.1.1 Chemicals 54
3.1.2 Animals 55
3.1.3 Experimental Design 55
3.1.4 *In silico* studies 56
3.1.5 Platelet and PRP isolation 58
3.1.6 *In vitro* platelet aggregation 58
3.1.7 Clot retraction 59
3.1.8 $FeCl_3$ induced carotid artery thrombosis 59
3.1.9 Bleeding and clotting time 60
3.1.10 *In vitro* thrombolytic activity 60
3.1.11 Statistical analysis 61
3.2 Results 61
3.2.1 DIM interacted with platelet GPVI receptors 61
3.2.2 DIM interacted with platelet P_2Y_{12} receptors 62
3.2.3 DIM exhibited low binding energy to GPVI and P_2Y_{12} receptors 64
3.2.4 DIM decreased *in vitro* platelet aggregation 65
3.2.5 DIM reduces clot retraction 67
3.2.6 DIM ameliorated the $FeCl_3$ induced TTO and thrombus weight 69
3.2.7 DIM improved cAMP and alleviated the COX-1, TXB_2, and PGE_2 in $FeCl_3$ induced thrombosis 69
3.2.8 DIM alleviated the platelet ROS and H_2O_2 72
3.2.9 DIM improved the bleeding and clotting time 73
3.2.10 DIM did not cause thrombolysis 74
3.3 Discussion 75
3.4 Summary 80

4 Introduction 83
4.1 Materials and methods 85
4.1.1 4.1.1 Experimental animals 85
4.1.2 Experimental design 85
4.1.3 MCAO surgery and drug treatment 86
4.1.4 Cerebral blood flow (CBF) measurement 87
4.1.5 Behavioral assessment 88
4.1.6 Brain infarction 88
4.1.7 Evaluation of blood-brain barrier integrity 88
4.1.8 Brain water content 89
4.1.9 Platelet aggregation 89
4.1.10 Platelet oxidative stress 89
4.1.11 ELISA 89
4.1.12 Histopathology 89
4.2 Results 90
4.2.1 DIM improved cerebral blood flow 90
4.2.2 DIM improved the neurobehavior in MCAO rats 92
4.2.3 DIM ameliorated brain infarction 93
4.2.4 DIM improved BBB integrity 94
4.2.5 DIM decreased brain water content 95
4.2.6 DIM inhibited platelet aggregation 96
4.2.7 DIM improved cAMP and inhibited COX-1, TXB_2, and PGE_2 97
4.2.8 DIM mitigated platelet oxidative stress 98
4.2.9 DIM alleviated inflammation 99
4.2.10 DIM ameliorated histopathological abnormalities 100

4.3 Discussion 102
4.4 Summary 107
5 Introduction 111
5.1 Materials & Methods 113
5.1.1 Animals 113
5.1.2 Chemicals 114
5.1.3 Experimental design Animal grouping 114
5.1.4 MCAO surgery 116
5.1.5 Assessment of neurobehavior 116
5.1.6 Measurement of Brain infarction 116
5.1.7 BBB integrity 116
5.1.8 Brain water content 116
5.1.9 Mitochondrial isolation 116
5.1.10 Measurement of mitochondrial complex enzyme activities 117
5.1.11 Mitochondrial membrane potential (MMP) and MPTP opening 117
5.1.12 ROS, LPO, SOD, and CAT 117
5.1.13 Enzyme-linked immunosorbent assay (ELISA) 118
5.1.14 RT-PCR 118
5.1.15 Histopathology 119
5.1.16 Statistical analysis 120
5.2 Results 120
5.2.1 I3C improved the neurobehavior in MCAO rats 120
5.2.2 I3C ameliorated the brain infarction 121
5.2.3 I3C alleviated the BBB leakages and brain water content 122
5.2.4 I3C improved the ETC complex enzyme activities 123

5.2.5 I3C improved the MMP and inhibited the MPTP opening 124
5.2.6 I3C mitigated the oxidative stress 125
5.2.7 I3C attenuated inflammation factors 126
5.2.8 I3C increased the protein levels of p-AMPK and PGC-1α 127
5.2.9 I3C increased mRNA levels of mitochondrial biogenesis markers 128
5.2.10 I3C altered apoptosis 130
5.2.11 I3C improved cortical architecture 131
5.3 Discussion 133
5.4 Summary 137
6 Summary and conclusion 141
6.1 Important outcomes 143
6.2 Future studies 144
6.3 Impact on the treatment of ischemic stroke 144