

**CEREBROPROTECTIVE EFFECTS OF INDOLE-3  
CARBINOL AND ITS MAJOR METABOLITE  
IN ISCHEMIC MODEL**



**Thesis submitted in partial fulfilment for the  
award of degree**

**Doctor of Philosophy**

**By**

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**July 2022**

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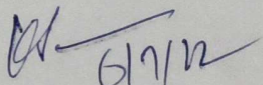
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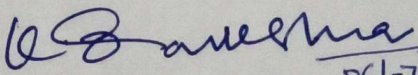
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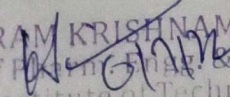
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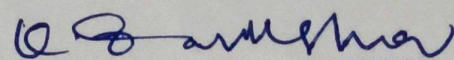
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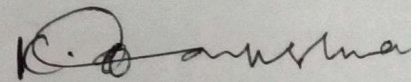
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(Kakarla Ramakrishna)

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## 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	<i>In silico</i> studies .....	56
3.1.5	Platelet and PRP isolation.....	58
3.1.6	<i>In vitro</i> platelet aggregation.....	58
3.1.7	Clot retraction .....	59
3.1.8	FeCl <sub>3</sub> induced carotid artery thrombosis.....	59
3.1.9	Bleeding and clotting time .....	60
3.1.10	<i>In vitro</i> 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 <sub>2</sub> Y <sub>12</sub> receptors .....	62
3.2.3	DIM exhibited low binding energy to GPVI and P <sub>2</sub> Y <sub>12</sub> receptors.....	64
3.2.4	DIM decreased <i>in vitro</i> platelet aggregation.....	65
3.2.5	DIM reduces clot retraction .....	67
3.2.6	DIM ameliorated the FeCl <sub>3</sub> induced TTO and thrombus weight.....	69
3.2.7	DIM improved cAMP and alleviated the COX-1, TXB <sub>2</sub> , and PGE <sub>2</sub> in FeCl <sub>3</sub> induced thrombosis.....	69
3.2.8	DIM alleviated the platelet ROS and H <sub>2</sub> O <sub>2</sub> .....	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 <sub>2</sub> , and PGE <sub>2</sub> .....	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-1 $\alpha$ .....	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

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## List of Figures

<b>Figure 1.1</b> Proposed hypothesis.....	14
<b>Figure 2.1</b> Objectives of the study.....	20
<b>Figure 2.2</b> Study design.....	20
<b>Figure 2.3</b> Chromatograms of rat plasma samples.....	31
<b>Figure 2.3.1</b> Plasma concentration of I3C and DIM in sham and MCAO rats.....	32
<b>Figure 2.4</b> I3C and DIM concentration in the brains of sham and MCAO rats.....	36
<b>Figure 2.5</b> I3C and DIM concentrations in CSF of sham and MCAO rats.....	38
<b>Figure 2.6</b> Effect of I3C treatment on neurological deficits.....	39
<b>Figure 2.7</b> Effect of I3C treatment on infarction size.....	40
<b>Figure 2.8</b> Effect of I3C treatment on blood-brain barrier permeability.....	41
<b>Figure 2.9</b> Effect of I3C treatment on brain water content.....	43
<b>Figure 3.1</b> The proposed hypothesis.....	51
<b>Figure 3.2</b> Study design.....	53
<b>Figure 3.3</b> Interaction of DIM with GPVI (2GI7).....	59
<b>Figure 3.4</b> Interaction of DIM with the P <sub>2</sub> Y <sub>12</sub> receptor.....	60
<b>Figure 3.5</b> Inhibitory concentrations of (IC <sub>50</sub> ) of DIM.....	62
<b>Figure 3.6</b> Effect of DIM treatment on in vitro platelet aggregation.....	63
<b>Figure 3.7</b> Effect of DIM treatment on thrombin-induced clot retraction.....	64
<b>Figure 3.8</b> Effect of DIM treatment on TTO and thrombus weight.....	65
<b>Figure 3.9</b> Effect of DIM treatment on cAMP, TXB <sub>2</sub> , COX-1, and PGE <sub>2</sub> levels.....	66

---

<b>Figure 3.10</b> Effect of DIM treatment on platelet ROS, H <sub>2</sub> O <sub>2</sub> , and NOX2 levels.....	68
<b>Figure 3.11</b> Effect of DIM treatment clotting and bleeding time.....	69
<b>Figure 3.12</b> The thrombolytic activity of DIM.....	70
<b>Figure 3.13</b> The summary of pharmacological activities of DIM against platelet aggregation and thrombosis.....	74
<b>Figure 4.1</b> Objective the study.....	78
<b>Figure 4.2</b> The experimental design.....	81
<b>Figure 4.3</b> Effect of DIM treatment on CBF in MCAO rats.....	84
<b>Figure 4.4</b> Effect of DIM treatment on MCAO-induced neurological deficits.....	85
<b>Figure 4.5</b> Effect of DIM treatment on brain infarction.....	86
<b>Figure 4.6</b> Effect DIM treatment on BBB permeability of EB.....	87
<b>Figure 4.7</b> Effect of DIM treatment on brain water content.....	88
<b>Figure 4.8</b> Effect of DIM treatment on platelet aggregation.....	89
<b>Figure 4.9</b> Effect of DIM treatment on cAMP, COX-1, TXB <sub>2</sub> , and PGE <sub>2</sub> levels.....	90
<b>Figure 4.10</b> Effect of DIM treatment platelet ROS and H <sub>2</sub> O <sub>2</sub> levels.....	91
<b>Figure 4.11</b> Effect of DIM treatment on inflammation.....	92
<b>Figure 4.12</b> Effect of DIM treatment on histopathological abnormalities in MCAO rats.....	93
<b>Figure 4.13</b> The pharmacological effects of DIM in MCAO rats.....	99
<b>Figure 5.1</b> The proposed hypothesis.....	104
<b>Figure 5.2</b> Study design.....	106
<b>Figure 5.3</b> Effect of I3C treatment on sensory-motor functions in MCAO rats.....	111

---

<b>Figure 5.4</b> The effect of I3C treatment on brain infarction.....	112
<b>Figure 5.5</b> The effect of I3C treatment on BBB integrity and brain water content.....	113
<b>Figure 5.6</b> The effect of I3C treatment on ETC complexes enzyme activities.....	114
<b>Figure 5.7</b> The effect of I3C treatment on MMP and MPTP opening.....	115
<b>Figure 5.8</b> The effect of I3C treatment on oxidative stress in MCAO rats.....	116
<b>Figure 5.9</b> The effect of I3C treatment on inflammation.....	117
<b>Figure 5.10</b> The effect of I3C treatment on protein levels of p-AMPK and PGC-1 $\alpha$ in MCAO rats.....	118
<b>Figure 5.11</b> The effect of I3C treatment on mRNA levels of PGC-1 $\alpha$ , NRF1, NRF2, and tFAM in MCAO rats.....	119
<b>Figure 5.12</b> The effect of I3C treatment on protein levels of cytochrome, caspase 9, and caspase 3 in MCAO rats.....	120
<b>Figure 5.13</b> The effect of I3C on histological changes after MCAO.....	122
<b>Figure 5.14</b> The mode of action of I3C against MCAO injury-induced pathological abnormalities.....	127
<b>Figure 6.1</b> Summary and conclusion of the study.....	132

---

## List of Tables

<b>Table 1.1</b> Risk factors of stroke.....	2
<b>Table 1.2</b> Limitation of current therapy in stroke.....	7.
<b>Table 2.1</b> Precision and accuracy of I3C and DIM.....	26
<b>Table: 2.2</b> Stability of I3C and DIM at different conditions.....	28
<b>Table 2.3</b> Recovery of I3C and DIM in plasma and other tissues.....	30
<b>Table 2.4</b> Pharmacokinetic parameters of I3C in MCAO rats.....	34
<b>Table 2.5</b> Tissue distribution of I3C and DIM.....	35
<b>Table 3.1</b> Binding energy of DIM with P <sub>2</sub> Y <sub>12</sub> receptors and GPVI receptors.....	61
<b>Table 4.1</b> Primer sequence of mitochondrial genes.....	110

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### List of Abbreviations

AA	: Arachidonic acid
AIS	: Acute ischemic stroke
ACN	: Acetonitrile
ADP	: Adenosine diphosphate
AMP	: Adenosine monophosphate
AMPK	: Adenosine monophosphate-activated protein kinase
ATP	: Adenosine triphosphate
ASP	: Aspirin
ANOVA	: Analysis of variance
AUC	: Area under the curve
BBB	: Blood-brain barrier
CAT	: Catalase
CBF	: Cerebral blood flow
CSF	: Cerebrospinal fluid
CLOP	: Clopidogrel
CT	: Computed tomography
CL	: Clearance
C <sub>max</sub>	: Maximum plasma concentration
Cyt. C	: Cytochrome C enzyme
DIM	: Diindolylmethane
DMSO	: Dimethyl sulfoxide
DNA	: Deoxy-ribonucleic acid
DCFH-DA	: Dichlorodihydrofluorescein diacetate
EB	: Evans blue

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ELISA	: Enzyme linked immunosorbant assay
ECA	: External carotid artery
FeCl <sub>3</sub>	: Ferric Chloride
FDA	: Food and Drug Administration
GAPDH	: Glyceraldehyde 3-phosphate dehydrogenase
HPLC	: High-performance liquid chromatography
H <sub>2</sub> O <sub>2</sub>	: Hydrogen peroxide
I3C	: Indole-3-carbinol
ICH	: International Council for Harmonisation
IL-6	: Interleukin-6
IL-10	: Interleukin-10
ICA	: Internal carotid artery (ICA)
I.V.	: Intravenous
ICZ	: Indolyl-carbazole
I/R	: Ischemic reperfusion
LLOD	: Lower limit of detection
LLOQ	: Lower limit of quantification
LGA	: Lamarckian Genetic Algorithm
MRI	: Magnetic resonance imaging
MCA	: Middle cerebral artery
MCAO	: Middle cerebral artery occlusion
MPTP	: Mitochondrial permeability transition pore (MPTP)
MMP	: Mitochondrial Membrane Potential
MB	: Mitochondrial biogenesis
MRT	: Mean residence time

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MDA	: Malonaldehyde
MgCl <sub>2</sub>	: Magnesium Chloride
NBT	: Nitroblue tetrazolium
NOX2	: Nicotine adenine dinucleotide phosphate oxidase 2
NRF1	: Nuclear respiratory factor
NRF2	: Nuclear respiratory factor
PGC1- $\alpha$	: Peroxisome proliferative activated receptor- $\gamma$ co-activator 1 $\alpha$
PBS	: Phosphate buffer saline
p-AMPK	: Phosphorylated-adenosine monophosphate-activated protein kinase
PDA	: Photodiode array
PK	: Pharmacokinetics
PD	: Pharmacodynamics
PRP	: Platelet-rich plasma
PPP	: Platelet-poor plasma
PGE <sub>2</sub>	: Prostaglandin E <sub>2</sub>
PMS	: Phenazine methosulphate
PDB	: Protein data bank
KCl	: Potassium chloride
ROS	: Reactive Oxygen Species
RtPA	: Recombinant tissue plasminogen activator
RSD	: Relative standard deviation
STAIR	: Stroke Therapy Academic Industry Roundtable
SD	: Standard deviation
SOD	: Superoxide dismutase
NaCl	: Sodium chloride

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NaHCO <sub>3</sub>	: Sodium bicarbonate
NaHPO <sub>4</sub>	: Sodium monohydrate phosphate
NADH	: Nicotine adenine dinucleotide phosphate
TMRM	: Tetramethylrhodamine methyl ester
TF	: Tissue factor
Tfam	: Mitochondrial transcriptional factor A
T <sub>max</sub>	: Time to reach the maximum plasma concentration
TTO	: Time to occlusion
TIA <sub>s</sub>	: Transient ischemic attacks
TXA <sub>2</sub>	: Thromboxane A <sub>2</sub>
TXB <sub>2</sub>	: Thromboxane B <sub>2</sub>
TTC	: Triphenyl tetrazolium chloride
T <sub>½</sub>	: Half-life
TNF- $\alpha$	: Tumor necrosis factor- $\alpha$
QC	: Quality control
V <sub>z</sub>	: Volume of distribution

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## **Preface**

The research work of the thesis entitled “Cerebroprotective effects of Indole-3-carbinol and its major metabolite in ischemic model” assessed the possible cerebroprotective mechanisms of indole-3-carbinol (I3C) and its metabolite diindolylmethane (DIM) for the treatment of ischemic stroke. Cerebral thrombosis as a result of atrial fibrillation or atherosclerosis is one of the leading cause of ischemic stroke. Ischemic stroke and reperfusion injury cause mitochondrial dysfunction, further aggravating brain injury. Therefore, we have evaluated the antiplatelet aggregation, antithrombotic, and mitochondrial protective mechanisms of I3C and DIM in ischemic stroke. The whole work has been compiled into six chapters: **Chapter 1** describes the introduction and significance of the present study. **Chapter 2** investigated the pharmacokinetics, brain penetration, and pharmacodynamics of I3C in focal cerebral ischemic rats. **Chapter 3** evaluated and compared the antiplatelet, antithrombotic, and thrombolytic activity of DIM with its parent compound, I3C. **Chapter 4** assessed the cerebroprotective role of DIM in focal cerebral ischemic injury in rats. **Chapter 5** determined the mitochondrial protective mechanisms of I3C in focal cerebral ischemic injury in rats. **Chapter 6** summarized the entire study completed with its essential outcomes.

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