

LIST OF CONTENTS

Chapter	Page
I INTRODUCTION	1
Rationale for the Study.....	1
Objectives of the study.....	4
Scope of the Study.....	5
Hypothesis.....	5
Anticipated outcomes.....	5
II LITERATURE REVIEW	6
Structure and function of blood vessel.....	6
Mediators involved in vasoconstriction and vasodilation.....	7
No and its role in vascular tone regulation.....	8
Mechanism of chemicals used as pharmacological tool in the present study.....	9
Rationale and background.....	11
Diagnosis and symptoms of PAH.....	11
Hemodynamics of pulmonary circulation.....	11
Pulmonary artery hypertension (PAH).....	13
Cause of PAH.....	15
Role of endothelium in controlling vascular contraction.....	16
Role of cGMP in contraction/relaxation.....	18
Current treatments for PAH.....	19
PDE5 Inhibitors.....	19
PGI ₂ Agonists.....	20
ET-1 Antagonists.....	21
<i>Curcuma longa</i> L.....	21
III RESEARCH METHODOLOGY	26
Materials	26
Preparations of compounds.....	26
Determination of PDE5 inhibitory activity.....	28

LIST OF CONTENTS (CONT.)

Chapter	Page
Determination of PDE6 inhibitory activity	29
Animals	29
Tissue preparation and vasorelaxation protocol	29
Statistical analysis	31
IV FINDINGS AND DISCUSSION	32
Inhibition of PDE5 and PDE6 by curcumin analogs	32
Vasorelaxation of pulmonary artery and aorta by curcuminoids	33
V CONCLUSIONS	37
Future perspectives	39
Conclusions	40
REFERENCES	41
APPENDIX	54
BIOGRAPHY	67

LIST OF TABLES

Table	Page
1 Percentage PDE5 inhibition of alcoholic extract of some Curcuma species tested at the final concentration (10 μ M).....	3
2 The inhibitory effect of 10 μ M curcumin and analogs on PDE5	4
3 Classification of pulmonary hypertension from WHO classification 2003	14
4 The inhibitory effects of curcumin and its analogues on PDE5 and PDE6. Values are means \pm S.E.M (n=3)	32
5 Vasorelaxant actions of curcumin and its analogues in endothelium-intact and denuded pulmonary arteries. (n=5-6), p-values indicated are for differences between endothelium-intact vs endothelium-denuded. # $p < 0.0001$ for endothelium-intact vs intact aorta.....	34

LIST OF FIGURES

Figure	Page
1 Structure of blood vessel	6
2 Mediators involved in vasoconstriction and vasodilation	7
3 Mechanism of ACh induced vasodilation	9
4 Mechanism of PE and high K ⁺ solution induced vasoconstriction	10
5 Pulmonary circulation	12
6 Nitric oxide pathway to increase cGMP for smooth muscle cell relaxation	16
7 Chemical structure of the PDE5 inhibitor structure of sildenafil and cGMP	20
8 Preparation of compounds 5–7. Reagents and conditions: (a) propargyl bromide, K ₂ CO ₃ , acetone, room temperature; (b) 20% aq. NaOH, EtOH, 0°C– room temperature.....	27
9 Preparation of compounds 8 and 9. Reagents and conditions: (a) NaN ₃ , H ₂ O, room temp, overnight; (b) CuSO ₄ , sodium ascorbate, THF:H ₂ O (9:1)	27
10 Mac Lab setting for evaluation of vasorelaxation effect	30
11 Time-course showing constriction of a endothelium-intact section of pulmonary artery the relaxant effect of compound 3 at 0.1 to 300 µM. PE=phenylephrine, ACh=acetylcholine, K= potassium.....	34
12 Concentration-vasorelaxation plots for the nine curcumin analogues and sildenafil. Each value is mean + SEM (n=5-6).* p< 0.05 compared relaxation of pulmonary arteries with and without endothelium, # p<0.05 comparing endothelium intact pulmonary arteries with aortas	36
13 Signaling pathway on smooth muscle relaxation and constriction	39
14 effect of compound 1 on pulmonary artery with endothelium (a), with out endothelium (b) and aorta (c)	55

LIST OF FIGURES (CONT.)

Figure	Page
15 effect of compound 2 on pulmonary artery with endothelium (a), without endothelium (b) and aorta (c).....	56
16 effect of compound 3 on pulmonary artery with endothelium (a), with out endothelium (b) a	57
17 effect of compound 4 on pulmonary artery with endothelium (a), without endothelium (b) and aorta (c)	58
18 effect of compound 5 on pulmonary artery with endothelium (a), without endothelium (b) and aorta (c)	59
19 effect of compound 6 on pulmonary artery with endothelium (a), without endothelium (b) and aorta (c)	60
20 effect of compound 7 on pulmonary artery with endothelium (a), without endothelium (b) and aorta (c).....	61
21 effect of compound 8 on pulmonary artery with endothelium (a), with out endothelium (b) and aorta (c).....	62
22 effect of compound 9 on pulmonary artery with endothelium (a), with out endothelium (b), and aorta (c)	63
23 effect of sildenafil on pulmonary artery with endothelium (a), without endothelium (b) and aorta (c).....	64
24 effect of DMSO 0.1% only on pulmonary artery with endothelium	65
25 Typical trace of contraction induced by 10^{-5} M PE after 45 min on pulmonary artery	65
26 Typical trace of contraction induced by 80 mM K^{+} on pulmonary artery	65
27 Typical trace of endothelium removed by 10^{-5} M ACh on pulmonary artery	66
28 Typical trace of relaxation responses by 10^{-5} M ACh and pre-contraction with 10^{-5} M PE on pulmonary artery	66

ABBREVIATIONS

PAH	=	pulmonary arterial hypertension
mm Hg	=	millimeter of mercury
NO	=	nitric oxide
eNOS	=	nitric oxide synthase
ET-1	=	endothelin-1
PGI ₂	=	prostacyclin
TXA ₂	=	thromboxane A ₂
PDE ₅	=	phosphodiesterase5
cGMP	=	guanosine 3', 5'-cyclic monophosphate
ATP	=	adenosine tri phosphate
BK	=	bradykinin
μM	=	micromolar
Ca ²⁺	=	calcium
CNP	=	C-natriuretic peptides
ACh	=	acetylcholine
5-HT	=	5-hydroxytryptamine
CAM	=	calmodulin
PIP ²	=	Phosphatidylinositol 4,5-bisphosphate
IP ³	=	inositol 1,4,5 triphosphate
ER	=	endoplasmic reticulum
GC	=	guanylyl cyclase
GTP	=	guanosine triphosphate
PKG	=	protein kinase G
SR	=	sarcoplasmic reticulum
SERCA	=	sarcoplasmic reticulum calcium adenosine tri phosphate
PE	=	phenylephrine
GPCR	=	Gq-protein couple receptor
MLCP	=	myosin light chain phosphorylation
DAG	=	diacylglycerol

ABBREVIATIONS (CONT.)

PKC	=	protein kinase C
K ⁺	=	potassium
H ₂ S	=	hydrogen sulfide
EDHF	=	endothelium derived hyperpolarizing factor
TNF-β	=	tumor necrosis factor-beta
PGF	=	prostaglandin-F
DMSO	=	dimethyl sulfoxide