## CONTENTS

<table>
<thead>
<tr>
<th>Abstract</th>
<th>Page no</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>i</td>
</tr>
<tr>
<td>Acknowledgement</td>
<td>ii- iv</td>
</tr>
<tr>
<td>Contents</td>
<td>iv-x</td>
</tr>
<tr>
<td><strong>Chapter I</strong></td>
<td>1-50</td>
</tr>
</tbody>
</table>

### GENERAL INTRODUCTION

Platelets: Role in Normal Physiology and Pathophysiology  
Effect of Conventional Therapeutic Drugs on Platelets  
Platelets and phytochemicals

Mechanism of apoptosis

- Morphological Modifications  
- Biochemical Events  
- Inducers of Apoptosis  
- The extrinsic pathway  
- The intrinsic pathway  
- Execution Pathway

Programmed cell death in platelets

Determination of platelet apoptosis

- Endogenously generated H$_2$O$_2$ 
- Mitochondrial inner membrane potential depolarization  
- Bcl2 protein family expression  
- Cytochrome c release from mitochondria 
- Caspase activation, a cytosolic marker of platelet apoptosis  
- Augmentation of cytosolic calcium  
- Mitochondrial permeability transition pore formation  
- Phosphatidylserine exposure  
- Platelet apoptotic markers at the whole-cell level  
- Platelet shrinkage  
- Morphological changes in apoptotic platelets 
- Upstream and downstream platelet apoptotic markers  
- Identification, characterization and quantification of platelet apoptosis in the lab

Mechanism of Platelet Aggregation  

25
Platelet-Derived Microparticle Generation

- Microparticles and coagulation
- Microparticles in hemostasis and thrombosis
- Crosstalk between Microparticles and Cells
- Role of Microparticles in Cardiovascular Diseases and Diabetes Mellitus
- Microparticles and Arthritis
- Microparticles and Cancer
- Microparticles and Infectious Diseases
- Role of Microparticles in Thromboembolism

Phytochemicals exhibiting pro-apoptotic effects on platelets
- Resveratrol
- Thymoquinone
- Gossypol
- Andrographolide

Phytochemicals that exert protective effects on platelet apoptosis
- Cinnamtannin B1

Concluding remarks

AIM AND SCOPE OF THE STUDY

Chapter II

CROCIN, A DIETARY ADDITIVE PROTECTS PLATELETS FROM OXIDATIVE STRESS-INDUCED APOPTOSIS AND INHIBITS PLATELET AGGREGATION

Introduction

- Materials and Methods
- Chemicals/Reagents
- Preparation of washed platelets
- Determination of endogenously generated ROS
- Determination of endogenously generated H₂O₂
- Estimation of intracellular calcium
- Determination of changes in mitochondrial membrane
<table>
<thead>
<tr>
<th>Chapter III</th>
<th>81-106</th>
</tr>
</thead>
</table>

SESAMOL INDUCES APOPTOSIS IN HUMAN PLATELETS VIA REACTIVE OXYGEN SPECIES-MEDIATED MITOCHONDRIAL DAMAGE

Introduction 81

Materials and Methods 82

- Chemicals/ Reagents 82
- Preparation of sesamol solution 83
- Preparation of washed platelets 83
- Determination of endogenously generated ROS 84
- Determination of endogenously generated $\mathrm{H}_2\mathrm{O}_2$ 85
- Estimation of total thiol, GSH and GSSG levels 85
- Estimation of intracellular calcium 85
- Determination of changes in mitochondrial membrane potential 86
- Preparation of platelet lysate 87
- Detection of cytochrome c release 87
- Assay of Caspases activity 87
- Determination of phosphatidylserine externalization 88
- Platelet aggregation 88
- Statistical analysis 88

Results 63

Discussion 66

Figures 73-80
Chapter IV

SESAMOL-INDUCED PLATELET APOPTOSIS: AMELIORATION BY CROCIN, A DIETARY COLORANT 107-130

Introduction 107

Materials and Methods 109

- Chemicals/Reagents 109
- Preparation of sesamol and crocin solutions 109
- Preparation of washed platelets 110
- Determination of endogenously generated ROS 110
- Determination of endogenously generated H$_2$O$_2$ 111
- Estimation of total thiol, GSH and GSSG levels 111
- Estimation of intracellular calcium 112
- Determination of changes in mitochondrial membrane potential 112
- Preparation of platelet lysate 113
- Detection of cytochrome c release 113
- Assay of Caspases activity 114
- Determination of phosphatidylserine externalization 114
- Statistical analysis 114

Results 115

Discussion 117

Tables 121-122

Figures 123-130

SUMMARY AND CONCLUSION 131-134

LIST OF ABBREVIATIONS 135-138

BIBLIOGRAPHY 139-155

LIST OF PUBLICATIONS 156-158
### LIST OF TABLES

**Chapter I**

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Inducers of Platelet Apoptosis</td>
<td>3</td>
</tr>
<tr>
<td>1.2</td>
<td>Differential action of phytochemicals on platelet functions</td>
<td>7-8</td>
</tr>
<tr>
<td>1.3</td>
<td>Normal range microparticles in circulation derived from different type of cells</td>
<td>28</td>
</tr>
</tbody>
</table>

**Chapter IV**

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Crocin inhibits sesamol-induced ROS and H$_2$O$_2$ generation, PS externalization and caspase activity</td>
<td>121</td>
</tr>
<tr>
<td>4.2</td>
<td>Crocin restores sesamol-induced depletion of GSH:GSSG ratio</td>
<td>122</td>
</tr>
<tr>
<td>4.3</td>
<td>Crocin ameliorates sesamol-induced intracellular Ca$^{2+}$ release and changes in ΔΨm</td>
<td>122</td>
</tr>
</tbody>
</table>

### LIST OF ILLUSTRATIONS

**Chapter I**

<table>
<thead>
<tr>
<th>Illustration</th>
<th>Title</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Schematic illustration of the general mechanism of intrinsic/mitochondria-mediated and extrinsic/death ligand-mediated apoptotic pathways in platelets</td>
<td>18</td>
</tr>
<tr>
<td>1.2</td>
<td>Schematic representation showing the release of microparticles from platelets and their pathophysiological effects</td>
<td>34</td>
</tr>
<tr>
<td>1.3</td>
<td>The proposed mechanisms for resveratrol and thymoquinone-induced platelet apoptosis.</td>
<td>45</td>
</tr>
<tr>
<td>1.4</td>
<td>The possible protective actions of cinnamtannin B1 (CTB) against platelet apoptosis.</td>
<td>50</td>
</tr>
</tbody>
</table>

**Chapter II**

<table>
<thead>
<tr>
<th>Illustration</th>
<th>Title</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Structure of Crocin</td>
<td>55</td>
</tr>
<tr>
<td>2.2</td>
<td>Effect of crocin on endogenous generation of ROS and H$_2$O$_2$</td>
<td>72</td>
</tr>
<tr>
<td>2.3</td>
<td>Effect of crocin on intracellular calcium release</td>
<td>73</td>
</tr>
<tr>
<td>2.4</td>
<td>Effect of crocin on caspase activity</td>
<td>74</td>
</tr>
<tr>
<td>2.5</td>
<td>Effect of crocin on mitochondrial membrane potential</td>
<td>75</td>
</tr>
<tr>
<td>2.6</td>
<td>Effect of crocin on phosphatidylserine externalization</td>
<td>76</td>
</tr>
</tbody>
</table>
2.7 Immunoblot of the effect of crocin on the expression of cytosolic cytochrome c 77
2.8 Effect of crocin on collagen-induced platelet aggregation and adhesion 78
2.9 The graphical abstract depicts the protective effect of crocin on platelets 79

Chapter III
3.1 Structure of Sesamol 82
3.2 Effect of sesamol on endogenous generation of ROS and H$_2$O$_2$ 97
3.3 Effect of sesamol on GSH:GSSG ratio and total thiols 98
3.4 Effect of sesamol on intracellular calcium release 99
3.5 Effect of sesamol on caspase activity 100
3.6 Effect of sesamol on mitochondrial membrane potential 101
3.7 Effect of sesamol on phosphatidylserine externalization 102
3.8 Immunoblot of the effect of sesamol on the expression of cytosolic cytochrome c 103
3.9 Effect of sesamol on agonist-induced platelet aggregation 104
3.10 A schematic representation depicting the proposed mechanism of the differential action of sesamol on platelets, at different concentrations 105

Chapter IV
4.1 Effect of crocin on sesamol-induced ROS generation 122
4.2 Effect of crocin on sesamol-induced H$_2$O$_2$ generation 123
4.3 Effect of crocin on sesamol-induced depletion of GSH:GSSG ratio and total thiol 124
4.4 Effect of crocin on sesamol-induced release of intracellular calcium 125
4.5 Effect of crocin on sesamol-induced caspase activation 126
4.6 Effect of crocin on sesamol-induced changes in mitochondrial membrane potential 127
4.7 Effect of crocin on sesamol-induced phosphatidylserine externalization 128
4.8 Immunoblot of the effect of crocin on sesamol-induced expression of cytosolic cytochrome c 129