CONTENTS

Declaration by the student ii
Certificate iii
Acknowledgements v
Abstract viii
Contents x
List of Figures xiv
List of Tables xvi
Abbreviations and Acronyms xvii

Chapter 1 Introduction 19

Chapter 2 Review of literature 22
  2.1 The Liver 22
    2.1.1 General structure 22
    2.1.2 Hepatocytes 23
    2.1.3 Functions of hepatocytes 23
    2.1.4 Biotransformation and detoxification 24
  2.2 Hepatotoxicity 25
    2.2.1 Hepatotoxicants 25
    2.2.2 APAP-induced hepatotoxicity 27
    2.2.3 In vitro toxicity 30
    2.2.4 In vitro toxicity models 30
    2.2.5 Hepatocellular carcinoma (HepG2) cell line as an in vitro
       hepatotoxicity model 32
  2.3 Assessment of drug-induced toxicity 34
2.3.1 Measurement of biochemical indices
2.3.2 Measurement of oxidative stress
2.3.3 DNA fragmentation, necrosis and apoptosis
2.4 Phytomedicine for hepatoprotection
2.5 Brassica juncea Czern. and Coss.: Possible role in hepatoprotection
2.6 Assessment of antioxidant activity of plants

Chapter 3 Objectives and plan of work

Chapter 4 Establishment of in vitro hepatotoxicity model
4.1 Introduction
4.2 Materials and methods
   4.2.1 Chemicals and reagents
   4.2.2 Maintenance of HepG2 cell line
   4.2.3 Characterization of HepG2 cell line
   4.2.4 Establishment of hepatotoxicity model on HepG2 cell line
   4.2.5 Statistical analysis
4.3 Results
   4.3.1 HepG2 cell line maintained morphological characteristics and expressed hepatic specific genes
   4.3.2 20 mM APAP-induced hepatotoxicity on HepG2 cell line
   4.3.3 Fluorescence microscopy revealed extensive cellular damage as a result of APAP-induced toxicity
   4.3.4 20 mM APAP-induced toxicity lead to extensive generation of ROS
   4.3.5 20 mM APAP-induced toxicity resulted in alteration of cell cycle pattern
4.4 Discussion

Chapter 5 Isolation and characterization of phytoconstituents from seeds of B. juncea
Section I: Pharmacognosy and physicochemical analysis
5.1 Introduction
5.2 Materials and methods
5.2.1 Chemicals and reagents 71
5.2.2 Sample collection and identification 71
5.2.3 Macroscopic and microscopic analysis 72
5.2.4 Physicochemical Characterization 72
5.3 Results 74
  5.3.1 Mustard seeds showed characteristic morphology 74
  5.3.2 Physicochemical properties of mustard seeds 74
Section II: Extraction and characterization of phytoconstituents 77
5.4 Introduction 77
5.5 Methods 77
  5.5.1 Preparation of plant extracts 77
  5.5.2 TLC autographic assay 79
  5.5.3 DPPH Radical Scavenging Activity 80
  5.5.4 Characterization of phytoconstituents 80
  5.5.5 Statistical analysis 84
5.6 Results 85
  5.6.1 Results of extraction of B. juncea seeds 85
  5.6.2 Characterization of BJHME 88
  5.6.3 B. juncea seeds showed presence of broad classes of phytoconstituents 88
Section III: Assessment of antioxidant activity of BJHME in vitro 101
5.7 Introduction 101
5.8 Methods 101
  5.8.1 FRAP assay 101
  5.8.2 Superoxide radical scavenging assay 102
  5.8.3 ABTS Radical Scavenging Assay 102
  5.8.4 ORAC-Hydrophillic Assay 103
5.9 Results 103
5.10 Discussion 104

Chapter 6 Cytotoxicity assessment and hepatoprotective efficacy testing of B. juncea seed extract 111
  6.1 Introduction 111
  6.2 Materials and methods 112
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.2.1 Chemicals and reagents</td>
<td>112</td>
</tr>
<tr>
<td>6.2.2 Maintenance of human dermal fibroblast (HDF) cell line</td>
<td>112</td>
</tr>
<tr>
<td>6.2.3 Assessment of BJHME toxicity on human dermal fibroblast-HDF cell line</td>
<td>113</td>
</tr>
<tr>
<td>6.2.4 Efficacy testing of BJHME on APAP-induced hepatotoxicity on HepG2 cell line</td>
<td>113</td>
</tr>
<tr>
<td>6.2.5 Elucidation of mechanism of hepatoprotection by BJHME against APAP-induced hepatotoxicity</td>
<td>115</td>
</tr>
<tr>
<td>6.2.6 Assessment of hepatoprotective effect of marker compounds detected in BJHME</td>
<td>117</td>
</tr>
<tr>
<td>6.2.7 Statistical analysis</td>
<td>117</td>
</tr>
<tr>
<td>6.3 Results</td>
<td>118</td>
</tr>
<tr>
<td>6.3.1 No toxicity seen of HDF cell line</td>
<td>118</td>
</tr>
<tr>
<td>6.3.2 Treatment with BJHME resulted in reversal of APAP-induced damage in vitro</td>
<td>118</td>
</tr>
<tr>
<td>6.3.3 Phytoconstituents present in BJHME showed in vitro hepatoprotective activity</td>
<td>128</td>
</tr>
<tr>
<td>6.4 Discussion</td>
<td>128</td>
</tr>
<tr>
<td><strong>Summary</strong></td>
<td>133</td>
</tr>
<tr>
<td><strong>References</strong></td>
<td>136</td>
</tr>
<tr>
<td><strong>Appendix I</strong></td>
<td>158</td>
</tr>
<tr>
<td><strong>Appendix II</strong></td>
<td>160</td>
</tr>
<tr>
<td><strong>List of Publications and presentations</strong></td>
<td>161</td>
</tr>
</tbody>
</table>