

CONTENTS

	Page No
ACKNOWLEDGEMENTS	i-iv
ABSTRACT	v-vii
LIST OF CONTENTS	viii-xxii
LIST OF TABLES	xxiii-xxviii
LIST OF FIGURES	xxix-xxxiii
CHAPTER 1 - INTRODUCTION AND REVIEW OF LITERATURE	1-63
1.1 Introduction and review of literature	2
1.1.1 Ayurveda and liver disease	6
1.1.1.a Complications	8
1.1.1.b Treatment	8
1.2 Botanical features and medicinal uses of <u>Melothria maderaspatana</u> and <u>Osbeckia octandra</u>	9
1.2.1 <u>Melothria maderaspatana</u>	12
1.2.2 <u>Osbeckia octandra</u>	15
1.3 Liver diseases	15
1.3.1 Classification of liver diseases	15
1.3.2 Jaundice and its classification	18
1.3.2.1 Post-hepatic jaundice	18
1.3.2.2 Hepatic jaundice	19
1.3.2.3 Pre-hepatic jaundice	20
1.3.2.4 Other rare varieties of jaundice	21

	Page No
1.4 Assessment of liver function	21
1.5 Plants with anti-hepatotoxic activity	25
1.5.1 Group I	26
1.5.1.1 Group Ia: In-vitro studies	26
1.5.1.2 Group Ib: In-vivo animal studies	26
1.5.2 Group II	26
1.5.2.1 Group IIa: In-vitro studies	26
1.5.2.2 Group IIb: In-vivo animal studies	26
1.5.3 Group III	26
1.5.4 Some active components and their chemical structures	38
1.5.4.1 Silymarin	38
1.5.4.2 Glycyrrhizin	40
1.5.4.3 (+)-Catechin	41
1.5.4.4 Schizandra lignoids	42
1.5.4.5 Saikosaporins	43
1.5.4.6 <u>Cynara scolymus</u>	44
1.5.5 Medicinal plants used in Sri Lanka for liver diseases	45
1.5.5.1 Plants used for liver congestion	46

	Page No
1.5.5.2 Plants used for liver inflammation	48
1.5.5.3 Plants used for jaundice	49
1.5.5.4 Plants used for various other liver diseases	56
1.5.6 New plant-based anti-hepatotoxic products.	60
CHAPTER 2 - MATERIALS AND METHODS	64-130
2.1 General	65
2.2 Experimental model	65
2.3 Preparation of drugs	65
2.3.1 <u>Melothria maderaspatana</u>	66
2.3.2 <u>Osbeckia octandra</u>	66
2.4 Dosage and administration of drugs	66
2.5 Induction of liver damage	67
2.6 Collection of blood for enzyme assays	67
2.7 Determination of serum alanine transaminase (ALT) E.C. 2.6.1.1 and serum aspartate transaminase (AST) E.C. 2.6.1.2	67
2.8 Determination of serum alkaline phosphatase activity E.C. 3.1.3.2	74
2.9 Methods used to determine the effect of the plant extracts on haematological parameters	77

	Page No
2.9.1 Determination of red blood cell count	77
2.9.2 Determination of white blood cell Count	79
2.9.3 Determination of packed cell Volume	81
2.10 Determination of reduced glutathione (GSH) in blood	83
2.11 Estimation of reduced glutathione (GSH) in the liver	86
2.12 Determination of total liver lipids	90
2.13 Determination of serum and liver cholesterol	92
2.13.1 Determination of serum cholesterol	97
2.13.2 Determination of liver cholesterol	97
2.14 Preparation of microsomal fraction of liver homogenate	99
2.15 Estimation of aminopyrine-N-demethylase	100
2.15.1 Determination of Michaelis-Menten constant (K_m) of aminopyrine-N- demethylase	104

	Page No
2.16 Estimation of aniline hydroxylase	105
2.16.1 Determination of Michaelis constant (K_m) of aniline hydroxylase	109
2.17 Estimation of proteins	110
2.17.1 Estimation of microsomal proteins, mitochondrial proteins or proteins in liver homogenate	114
2.18 Measurement of phenylbarbital- induced loss of righting reflex	115
2.19 Preparation of mitochondrial fraction of the liver homogenate	116
2.20 Estimation of acid phosphatase E.C. 3.1.3.2 in mitochondrial fraction	116
2.21 Estimation of β -glucuronidase E.C. 3.2.1.31	121
2.22 Estimation of lipid peroxidation	124
2.24 Methods used to determine the effect of plant extract on reproductive activity	127
2.24.1 Preparation of vaginal smears	127
2.24.2 Effect of plant extracts on ovulatory activity	128
2.24.3 Effect of plant extracts on implantation activity	128
2.24.4 Effect of the plant extracts on early abortifacient activity	129

	Page No
2.24.5 Effect of plant extracts on spermatogenic activity	130
CHAPTER 3 - A PRELIMINARY INVESTIGATION OF THE ANTI-HEPATOTOXIC ACTIVITY OF <u>M.MADERASPATANA</u> AND <u>O.OCTANDRA</u>	131-173
3.1 Introduction	132
3.2 Experimental	134
3.2.1 Experimental animals	134
3.2.2 Preparation of plant extracts	135
3.2.3 Dosage and administration of drugs	135
3.2.4 Effect of extracts of <u>M.maderaspatana</u> or <u>O.octandra</u> on carbon tetrachloride induced liver dysfunction	135
3.2.5 Effect of the extracts of <u>M.maderaspatana</u> and <u>O.octandra</u> on ethanol induced hepatic damage	136
3.2.6 Effect of storage of the extracts of <u>M.maderaspatana</u> and <u>O.octandra</u> on CCl ₄ induced hepatic damage	137
3.2.7 Seasonal variations of <u>M.maderaspatana</u> and <u>O.octandra</u> on carbon tetrachloride induced liver damage	139

	Page No
3.2.8 Effect of dosage	139
3.2.9 A comparison of the anti-hepatotoxic activities of <u>M.maderaspatana</u> and <u>O.octandra</u> with that of (+)3-Cyanidanol, an established anti-hepatotoxic agent	139
3.3 Results	140
3.3.1 Effect of the extracts of <u>M.maderaspatana</u> and <u>O.octandra</u> on carbon tetrachloride induced liver dysfunction	140
3.3.2 Effect of the extracts of <u>M.maderaspatana</u> and <u>O.octandra</u> on ethanol induced hepatic damage	142
3.3.3 Effect of storage of extracts of carbon tetrachloride induced hepatotoxicity	143
3.3.4 Seasonal variations of <u>M.maderaspatana</u> or <u>O.octandra</u> on carbon tetrachloride induced hepatotoxicity	144
3.3.5 Effect of dosage of <u>M.maderaspatana</u> and <u>O.octandra</u> on carbon tetrachloride induced hepatotoxicity	145

	Page No
3.3.6 A comparison of <u>M.maderaspatana</u> <u>O.octandra</u> and (+)-3-cyanidanol as anti-hepatotoxic agents	145
3.4 Conclusion	147
CHAPTER 4 - MECHANISM OF ACTION OF <u>MELOTHRIA</u> <u>MADERASPATANA</u> AND <u>OSBECKIA OCTANDRA</u> AGAINST CARBON TETRACHLORIDE INDUCED LIVER DAMAGE	
	174-208
4.1 Introduction	175
4.2 Experimental	177
4.2.1 Effect of <u>M.maderaspatana</u> and <u>O.octandra</u> on carbon tetrachloride induced changes in hepatic drug metabolizing enzymes: Aniline hydroxylase and p-Aminopyrine-N -demethylase	177
4.2.1.1 Effect of the extracts of <u>M.maderaspatana</u> and <u>O.octandra</u> on the K_m value of the microsomal enzymes aniline hydroxylase and p-aminopyrine-N- demethylase	179

- 4.2.1.2 Effect of M.maderaspatana and O.octandra extracts on carbon tetrachloride mediated alterations in the rats of phenobarbital induced loss of righting reflex 180
- 4.2.2 Effect of M.maderaspatana and O.octandra on carbon tetrachloride induced changes in rat liver lysosomes 181
- 4.2.3 Effect of M.maderaspatana and O.octandra on lipid peroxidation induced by carbon tetrachloride 182
- 4.2.4 Effect of M.maderaspatana and O.octandra on carbon tetrachloride induced changes in blood and liver glutathione levels 183
- 4.2.5 Effect of the aqueous extracts of M.maderaspatana and O.octandra on the immune system 184
- 4.2.5.1 Preparation of plant material for immunological studies 184

4.2.5.2 Complement tests

4.2.5.2.1 Haemolytic assay

of human

complement activity 185

4.3 Results

4.3.1 The effects of carbon tetrachloride, M.maderaspatana and O.octandra on the activities on hepatic microsomal enzymes, aniline hydroxylase and p-aminopyrine-N-demethylase 186

4.3.1.1 Effects of the extracts of M.maderaspatana and O.octandra on the K_m values of microsomal enzymes aniline hydroxylase and p-aminopyrine-N-demethylase 188

4.3.1.2 Effect of M.maderaspatana and O.octandra on carbon tetrachloride mediated alterations of phenobarbital induced righting reflex in rats. 188

	Page No
4.3.2 Effect of <u>M.maderaspatana</u> and <u>O.octandra</u> on carbon tetrachloride induced changes in rat liver lysosomes	189
4.3.2.1 Acid phosphatase activity	189
4.3.2.2 β -Glucuronidase activity	190
4.3.3 Effect of <u>M.maderaspatana</u> or <u>O.octandra</u> extracts on carbon tetrachloride induced melondialdehyde formation in rat liver	190
4.3.4 Effect of <u>M.maderaspatana</u> and <u>O.octandra</u> on carbon tetrachloride induced changes in reduced glutathione levels in blood and liver	191
4.3.5.1 Effect of complement activity	191
4.3.5.2 Effects on chemiluminescence of polymorphonuclear leucocytes	192
4.4 Conclusion	192
CHAPTER 5 - STUDIES OF THE TOXICITY OF <u>MELOTHRIA MADERASPATANA</u> AND <u>OSBECKIA OCTANDRA</u>	
	209-243
5.1 Introduction	210
5.2 Experimental	211

	Page No
5.2.1 Effect of the extracts of <u>M.maderaspatana</u> and <u>O.octandra</u> on liver function and histopathology of various organs	211
5.2.2 Effect of the extracts of <u>M.maderaspatana</u> and <u>O.octandra</u> on red blood cell count, white blood cell count, packed cell volume and haemoglobin concentration	212
5.2.3 Effect of <u>M.maderaspatana</u> and <u>O.octandra</u> on implantation activity	213
5.2.4 Effect of the extracts of <u>M.maderaspatana</u> and <u>O.octandra</u> on early abortifacient activity	213
5.2.5 Effect of the extracts of <u>M.maderaspatana</u> and <u>O.octandra</u> on ovulatory activity	214
5.2.6 Effect of <u>M.maderaspatana</u> and <u>O.octandra</u> on anti-spermatogenic activity	214
5.3 Results	
5.3.1 Effect of the aqueous extracts of <u>M.maderaspatana</u> and <u>O.octandra</u> on liver function	215

	Page No
5.3.2 Effects of the extracts of <u>M.maderaspatana</u> and <u>O.octandra</u> on the histopathology of various internal organs	215
5.3.2.1 Liver	216
5.3.2.2 Kidney	216
5.3.2.3 Lung	217
5.3.2.4 Heart	217
5.3.2.5 Intestine	218
5.3.2.6 General observations	218
5.3.3 Effect of the extracts of <u>M.maderaspatana</u> and <u>O.octandra</u> on red blood cell count, white blood cell count, packed cell Volume and haemoglobin concentration	219
5.3.4 Effect of the aqueous extracts of <u>M.maderaspatana</u> and <u>O.octandra</u> on implantation activity	220
5.3.5 Effect of aqueous extracts of <u>M.maderaspatana</u> and <u>O.octandra</u> on early abortifacient activity	221
5.3.6 Effect of the aqueous extracts of <u>M.maderaspatana</u> and <u>O.octandra</u> on ovulatory activity	221

	Page No
5.3.7 Effect of <u>M.maderaspatana</u> and <u>O.octandra</u> on spermatogenic activity	222
5.4 Conclusion	222
CHAPTER 6 - ISOLATION OF ACTIVE COMPONENTS FROM <u>M.MADERASPATANA</u> AND <u>O.OCTANDRA</u>	244-297
6.1 Introduction	245
6.2 Materials and methods	245
6.2.1 Reagents	245
6.2.2 Screening of alkaloids	247
6.2.3 Screening for saponins	248
6.2.3.1 Froth test	248
6.2.3.2 Haemolysis test	249
6.2.4 Screening of steroids/ triterpenoids	
6.2.4.1 Salkowski test	249
6.2.5 Screening for cardiac glycosides	250
6.2.5.1 Kedde test	250
6.2.5.2 Keller-Killini test	251
6.2.6 Screening for cannabinoids	251
6.2.7 Screening for flavonoids	252
6.3 Isolation of active components from <u>M.maderaspatana</u>	253
6.3.1 Separation of active fractions	253

	Page No
6.3.2 Separation of components in the active fractions	254
6.3.2.1 The petroleum ether fraction	254
6.3.2.2 The dichloromethane fraction	254
6.3.2.3 The ethylacetate fraction	255
6.3.2.4 The methanol fraction	255
6.3.2.5 The aqueous fraction	256
6.4 Biological testing	256
6.5 Results	257
6.6 Conclusion	258
6.7 <u>Osbeckia octandra</u>	259
6.7.1 Separation of active fractions	259
6.7.2 Separation of components in active fractions	260
6.7.2.1 Ethyl acetate fraction	260
6.7.2.2 The aqueous fraction	261
6.8 Biological testing	261
6.9 Results	262
6.10 Conclusion	265
CHAPTER 7 - DISCUSSION	298-312
REFERENCES	313-328
PUBLICATIONS	329

List of Tables

	Page No	
Table 1	Morphologic classification of liver diseases	16-17
Table 2	Liver function tests	22-25
Table 3	Plants with confirmed antihepatotoxic activity. In-vitro studies	27-28
Table 4	Plants with confirmed antihepatotoxic activity. In-vivo animal studies	29
Table 5	Plants with proven antihepatotoxic activity and active principle isolated. In-vitro studies	30-31
Table 6	Plants with proven antihepatotoxic activity and active principle isolated. In-vivo animal studies	33-37
Table 7	Clinical trials carried out on human patients using the isolated active principles	37-38
Table 8	Plant based antihepatotoxic drugs sold in the market	61-63
Table 9	Assay medium for ALT calibration graph	71
Table 10	Assay medium for AST calibration graph	74
Table 11	Preparation of reduced glutathione (GSH) standard curve	86

	Page No
Table 12 Assay medium for serum cholesterol	94
Table 13 Assay medium for the preparation of standard curve for serum cholesterol	95
Table 14 Aminopyrine-N-demethylase assay medium	103
Table 15 Assay medium for the determination of K_m value of aminopyrine-N-demethylase	105
Table 16 Assay medium for aniline hydroxylase	107
Table 17 Assay medium for the determination of K_m value of aniline hydroxylase	109
Table 18 Assay medium for the preparation of standard curve for protein determination	112
Table 19 Assay medium for the determination of malondialdehyde	125
Table 20 Effect of <u>M.maderaspatana</u> or <u>O.octandra</u> post-treatment on carbon tetrachloride induced hepatotoxicity	149
Table 21 Effect of <u>M.maderaspatana</u> or <u>O.octandra</u> pretreatment on carbon tetrachloride induced hepatotoxicity	150
Table 22 Effect of <u>M.maderaspatana</u> and <u>O.octandra</u> extracts on ethanol induced hepatotoxicity	158
Table 23 Effect of storage of the extracts of <u>M.maderaspatana</u> and <u>O.octandra</u> on CCl_4 induced hepatotoxicity	167

Table 24	Effect of seasonal variations of <u>M.maderaspatana</u> and <u>O.octandra</u> CCl ₄ induced hepatotoxicity	168
Table 25	Effect of dosage of <u>M.maderaspatana</u> and <u>O.octandra</u> on CCl ₄ induced hepatotoxicity	169
Table 26	Effect of <u>M.maderaspatana</u> , <u>O.octandra</u> or (+)-3-cyanidanol post-treatment on CCl ₄ induced hepatotoxicity	170
Table 27	The effects of <u>M.maderaspatana</u> , <u>O.octandra</u> or (+)-3-cyanidanol pre-treatment on CCl ₄ induced hepatotoxicity	171
Table		
28.1	Effect of CCl ₄ on aniline hydroxylase activity and p-aminopyrine-N-demethylase activity	194
Table		
28.2	Effect of <u>M.maderaspatana</u> and <u>O.octandra</u> extracts on CCl ₄ mediated alterations in rats of phenobarbital induced loss of righting reflex	201
Table 29	Effect of <u>M.maderaspatana</u> or <u>O.octandra</u> on CCl ₄ mediated alterations in rat liver lysosomes	202

Table 30	Effect of <u>M.maderaspatana</u> or <u>O.octandra</u> on CCl ₄ induced malondialdehyde formation in rat liver	203
Table 31	Effect of <u>M.maderaspatana</u> and <u>O.octandra</u> on CCl ₄ induced changes in blood and liver glutathione levels	204
Table 32	Comparison of the specific activities of <u>M.maderaspatana</u> and <u>O.octandra</u>	205
Table 33	Effect of the aqueous extracts of <u>M.maderaspatana</u> and <u>O.octandra</u> on serum levels of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase	223
Table 34	Effect of the aqueous extracts of <u>M.maderaspatana</u> or <u>O.octandra</u> on red blood cell count (RBC), white blood cell count (WBC), packed cell volume (PCV) and haemoglobin concentration (Hb%)	239
Table 35	Effect of the aqueous extracts of <u>M.maderaspatana</u> or <u>O.octandra</u> on implantation activity	240
Table 36	Effect of the extracts of <u>M.maderaspatana</u> or <u>O.octandra</u> on early abortifacient activity	241

	Page No
Table 37 Effect of the extracts of <u>M.maderaspatana</u> or <u>O.octandra</u> on ovulatory activity	242
Table 38 Effect of the extracts of <u>M.maderaspatana</u> or <u>O.octandra</u> on spermatogenic activity	243
Table 39 The effects of different fractions of <u>M.maderaspatana</u> on CCl ₄ induced hepatotoxicity	266
Table 40 Chemical screening of different fractions of <u>M.maderaspatana</u>	267
Table 41 Effects of alkaloids (A,B and C) separated from the methanol fraction, on CCl ₄ induced hepatotoxicity	268
Table 42 The effects of the crude methanol extract of <u>O.octandra</u> and different fractions on CCl ₄ induced hepatotoxicity	280
Table 43 Chemical screening of different fractions of <u>O.octandra</u>	281
Table 44 The effects of the components A,B and C isolated from the ethyl acetate and aqueous fractions of <u>O.octandra</u> on CCl ₄ induced hepatotoxicity	282

	Page No
Table 45 Effects of flavonoids (A_1 , A_2 and A_3) separated from compound A on CCl_4 induced hepatotoxicity	283

List of Figures

	Page No
Fig 1	<u>Melothria maderaspatana</u> plant 11
Fig 2	<u>Osbeckia octandra</u> plant 14
Fig 3	Standard curve for the assay of serum alanine transaminase 72
Fig 4	Standard curve for the assay of serum aspartate transaminase 73
Fig 5	Standard curve for the assay of reduced glutathione 89
Fig 6.1	Standard curve for the assay of serum cholesterol 96
Fig 6.2	Standard curve for the assay of liver cholesterol 98
Fig 7	Standard curve for protein determination 113
Fig 8A- Fig 8G	Histopathological evidence for the protective effect of <u>M.maderaspatana</u> and <u>O.octandra</u> on CCl ₄ induced liver damage (8A-8G) 151-157
Fig 9A- Fig 9G	Histopathological evidence for the protective effect of <u>M.maderaspatana</u> or <u>O.octandra</u> on ethanol induced liver damage 160-166

	Page No
Fig 10A- Fig 10 B	172-173
Fig 11	195
Fig 12	197
Fig 13	199
Fig 14	205
Fig 15	207

Fig 16	Reduction of luminol induced chemiluminescence of human polymorphonuclear cells upon stimulation with zymosan by increasing doses of <u>O.octandra</u> and <u>M.maderaspatana</u>	208
Fig 17	A: Liver of rat after 7 days of oral administration of distilled water	224
	B: Liver of rat after 7 days of oral administration of <u>M.maderaspatana</u> extract	225
	C: Liver of rat after 7 days of oral administration of <u>O.octandra</u> extract	226
Fig 18	A: Kidney of rat after 7 days of oral administration of distilled water	227
	B: Kidney of rat after 7 days of oral administration of <u>M.maderaspatana</u>	228
	C: Kidney of rat after 7 days of oral administration of <u>O.octandra</u>	229
Fig 19	A: Lung of rat after 7 days of oral administration of distilled water	230
	B: Lung of rat after 7 days of oral administration of <u>M.maderaspatana</u>	231
	C: Lung of rat after 7 days of oral administration of <u>O.octandra</u> extract	232

	Page No	
Fig 20	A: Heart muscles of rat after 7 days of oral administration of distilled water.	233
	B: Heart muscles of rat after 7 days of oral administration of <u>M.maderaspatana</u> extract.	234
	C: Heart muscles of rat after 7 days of oral administration of <u>O.octandra</u> extract.	235
Fig 21	A: Intestine of rat after 7 days of oral administration of distilled water	236
	B: Intestine of rat after 7 days of oral administration of <u>M.maderaspatana</u> extract.	237
	C: Intestine of rat after 7 days of oral administration of <u>O.octandra</u> extract	238
Fig 22A- Fig 22G	Histopathological evidence for the antihepatotoxic effects of different fractions of <u>M.maderspatana</u> on CCl ₄ induced hepatotoxicity(Fig 22A-Fig 22G)	269-275
Fig 23	Diagrammatic representation of the components observed on chromatographic separation of the methanol fraction of <u>M.maderaspatana</u>	276

	Page No
Fig 24A - Histopathological evidence for the Fig 24 C anti-hepatotoxic effects of the alkaloids A,B and C isolated from the methanol fraction of <u>M.maderaspatana</u> . (Fig 24A-Fig24C)	277-279
Fig 25A- Histopathological evidence for the Fig 25 G effects of different fractions isolated from <u>O.octandra</u> (Fig 25A-Fig 25G)	284-290
Fig 26 Diagrammatic representation of the components observed on chromatographic separation of compound A from the ethyl acetate fraction of <u>O.octandra</u>	291
Fig 27A- Histopathological evidence for the Fig 27 C anti-hepatotoxic effects of flavonoids A,B and C isolated from ethylacetate and aqueous fraction of <u>O.octandra</u> (Fig 27A-Fig 27C)	292-294
Fig 28A- Histopathological evidence for the Fig 28 C anti-hepatotoxic effects of flavonoids and flavonoid derivatives A ₁ ,A ₂ and A ₃ isolated from fraction A of ethylacetate and aqueous fractions of <u>O.octandra</u>	295-297