CONTENTS

Acknowledgements i
Abbreviations ii-iii

CHAPTER 1 INTRODUCTION 1-15

1.1 Historical account 1
1.2 Definition of the problem and risk factors 1
1.3 Types of leishmaniasis 2
1.4 Geographical distribution of leishmaniasis 5
1.5 Visceral leishmaniasis: global status 6
1.6 Visceral leishmaniasis: national status 6
1.7 Leishmania/HIV co-infection 7
1.8 Clinical symptoms of VL 8
1.9 Life cycle of Leishmania donovani 9
1.10 Causative agent 9
1.11 Vector and transmission of the disease 10
1.12 Control strategies 11
1.13 Diagnosis of VL 11
1.14 Vaccines against kala-azar 12
1.15 Drugs against kala-azar 13
1.16 Rationale of the study 13
1.17 Aims and objectives in the undertaken work 15

CHAPTER 2 REVIEW OF LITERATURE 16-36

2.1 Current scenario of antileishmanials for VL 16
2.2 Drug resistance in Leishmania: current scenario, reasons and mechanisms 19
2.3 Medicinal plants as potential source of antileishmanials 20
2.4 Marine products as potential source of antileishmanials 22
2.5 Screening models for antileishmanial testing 23
  2.5.1 In vitro screening systems 23
  2.5.2 Animal models for screening of antileishmanial screening 25
2.6 Current in vitro assays for antileishmanial screening 28
  2.6.1 Cell viability assays 28
  2.6.2 Fluorescence dye based assay 30
  2.6.3 Ornithine decarboxylase (ODC) based assay 30
  2.6.4 Reporter-gene based screening assay 30
2.7 Applications of GFP in antileishmanial screening 32
2.8 Other biological studies with transgenic GFP-parasites 35
2.9 Animal models developed using reporter genes 36
CHAPTER 3 MAINTENANCE OF LEISHMANIA 37-41

3.1 Preparation of culture media 37
3.2 Experimental host for Leishmania 37
3.3 In vitro maintenance 39
3.4 In vivo maintenance 40

CHAPTER 4 TRANSFECTION OF LEISHMANIA AND EXPRESSION OF GFP 42-65

4.1 Introduction 42
4.2 Episomal expression of GFP in Leishmania 43
4.3 Stable expression of GFP in Leishmania 52
4.4 Discussion 63

CHAPTER 5 IN VITRO INFECTION STUDIES WITH GFP TRANSFECTANTS 66-83

5.1 Infectivity of GFP-transfectants towards macrophages in vitro 66
5.1.1 In vitro infectivity of episomal transfectants 68
5.1.2 In vitro infectivity of stable transfectants 74
5.2 Generation of axenic amastigotes of stable transfectants 76
5.3 Discussion 81

CHAPTER 6 DEVELOPMENT OF GFP-BASED ANTILEISHMANIAL DRUG SCREENING ASSAYS 84-106

6.1 Materials and methods 84
6.2 Results 88
6.2.1 Assessment of efficacy of standard drugs against episomal and stable transfectants by FACS 88
6.2.2 Assessment of efficacy of standard drugs against episomal transfectants by microplate fluorometric assay 96
6.2.3 Assessment of efficacy of standard drugs against stable transfectants by microplate fluorometric assay 97
6.3 Discussion 100

Tables 6.1 to 6.3 104-106

CHAPTER 7 APPLICATIONS OF GFP-BASED ASSAYS IN DISCOVERY OF NEW ANTILEISHMANIALS FROM NATURAL RESOURCES 107-122

7.1 Materials and methods 107
7.2 Results 108
7.2.1 In vitro activity of plant and marine samples 108
7.2.2 In vivo efficacy of plant and marine samples 114
7.2.2.1 Desmodium gangeticum 114
7.2.2.2 Dyssoxylum binectariferum 115
7.2.2.3 Piper betle 116
7.2.2.4 Tinospora sinensis 117
7.2.5 Haliclona exigua
7.2.6 Actinopyga lecanora
7.2.3 Correlation between in vitro activity obtained in FACS assay and in vivo activity in hamsters
7.3 Discussion

CHAPTER 8 IN VIVO INFECTION STUDIES WITH GFP TRANSFECTANTS 123-127

8.1 Materials and methods 123
8.2 Results 124
8.3 Discussion 126

SUMMARY 128-133
BIBLIOGRAPHY 134-151
APPENDICES 152-153
RESEARCH PUBLICATIONS 154