TABLE OF CONTENTS

TOPICS P. No’s

List of Tables i-iv
List of Figures v-xii
Abbreviations xiii-xiv

1. INTRODUCTION 1-10

2. REVIEW OF LITERATURE 11-30
   2.1 Concept of Stress
      2.1.1 Physiology of stress response
      2.1.2 The hypothalamic-pituitary-adrenal axis
   2.2 Immune System 16
      2.2.1 Innate Immunity
      2.2.2 Adaptive Immunity
      2.2.3 Effectors of the Immune System
   2.3 Stress related disorders 24
      2.3.1 Stress induced immunological alterations
      2.3.2 Stress induced biochemical and behavioral alterations
   2.4 Stress and wound healing 27
   2.5 Herbals as stress busting agents 28

3. MATERIALS AND METHODS 31-68
   3.1 Chemicals and instruments used 32
   3.2 Plant material 34
      3.2.1 Plant1: Cicer microphyllum
         3.2.1.1 Preparation of extracts
         3.2.1.2 Fractionation of 13 M/38A001
      3.2.2 Plant2: Taraxacum officinale
         3.2.2.1 Preparation of extracts
         3.2.2.2 Standardization of TO-10 by High Performance Thin Layer Chromatography (HPTLC)
         3.2.2.3 Fractionation of TO-10
         3.2.2.4 Isolation of chicoric acid
         3.2.2.5 HPLC of chicoric acid
3.3 Acute Toxicity Studies 44

3.4 Animals 44

3.5 Antigenic stimulus 44

3.6 Experimental design 44

3.6.1 IMMUNOLOGICAL PARAMETERS 45

3.6.1.1 Immunomodulatory evaluation of the test material 45

a) Primary screening 45

(a1) Estimation of TNF-alpha in LPS activated murine neutrophils

(a2) Comparative evaluation of test material on humoral and cell mediated immune response

(a2.1) Humoral immune response

(a2.2) Delayed Type Hypersensitivity (DTH) response.

(a2.3) Estimation of markers and Th1 and Th2 cytokines.

b) Multiple dose immunomodulatory study of the most active extracts 48

(13M/38/A001 and TO-10)

In-vitro studies

(b1) Lymphocyte Proliferation Assay

(b2) Phagocytic response in-vitro and in-vivo.

(b3) Estimation of Th1/Th2 cytokines expression in (PMA+I) stimulated murine PBMC

In-vitro studies

(b4) In normal SRBCs immunized mice 50

(b4.1) Delayed type hypersensitivity response

(b4.2) Estimation of T-cell surface markers.

(b4.3) Flowcytometric estimation of CD4\(^+\) IL-2, IFN-gamma and IL-4 expression.

(b4.4) Flow cytometric analysis of co stimulatory molecules.

c) Immunomodulatory studies in Cyclosporine-A induced immune-suppressed animals. 52

(c1) Estimation of T cell surface markers and Th1/Th2 cytokines.

(c2) Graft rejection time (days) in Cyclosporine-A induced immune-suppressed mice
Vascular permeability in mice subjected to chemical induced immune-suppression.

3.6.1.2 Antistress evaluation of active extracts (I^3M/38/A001 and TO-10)

a) Chronic restraint stress
   (a1) Method of stress induction
   (a2) During administration
   (a3) Lymphocyte Immunophenotyping
   (a4) Estimation of CD244^NK cells population
   (a5) Intracellular cytokine estimation
   (a6) Estimation of polymorphonuclear leucocytes count.
   (a7) Corticosterone assay

b) SRBCs immunization in chronically restraint stressed mice
   (b1) Flowcytometric analysis of T cell surface markers and Th1/Th2 cytokines in SRBC immunized chronically stressed mice
   (b2) Quantification of cytokines in spleen cell culture supernatant.
   (b3) Flowcytometric analysis of co-stimulatory molecules.
   (b4) Delayed Type hypersensitivity response in stressed animals.
   (b5) Corticosterone assay
   (b6) Body and Organs weight ratio.

c) Chronic cold stress
   (c1) Method of stress induction and drug administration
   (c2) Estimation of total and differential leukocyte count
   (c3) Flowcytometric estimation of Th1/Th2 cytokines in splenocytes (ex-vivo)

3.6.1.3 Identification of the active fractions

a) Immunomodulatory screening
   (a1) Effect of test fractions on intracellular IL-2 expression in PMA + I stimulated murine PBMCs.
   (a2) Comparative evaluation of the effect of active fractions on humoral and cell mediated immuneresponses.
   (a3) Estimation of T and B cell surface markers and Th1 and Th2 cytokines
b) Antistress screening  
(b1) Effect on T and B cell surface markers.  
(b2) Corticosterone assay  
c) Detailed Investigation of the antistress activity of the active fraction C.F.  

3.6.1.4 Identification of the active principle  
3.6.2 BIOCHEMICAL PARAMETERS  
a) Stress induction and drug administration  
b) Estimation of biochemical parameters in adrenal glands  
c) Estimation of hepatic parameters.  
d) Estimation of biochemical parameters in serum  

3.6.3 CHRONIC STRESS INDUCED BEHAVIOURAL DEPRESSION  
a) Estimation of neurotransmitters in brain region  

3.6.4 CHRONIC STRESS INDUCED DELAYED WOUND HEALING  
a) Restraint stress paradigm and wound induction  
b) Estimation of cytokines at the wound site  

3.6.5 OTHER STUDIES  
a) Antifatigue effect  
b) Swimming endurance test  
c) Hypoxia test  

4. RESULTS  

SECTION-A: Preliminary Screening of Potential immunodulators  
SECTION-B: Multiple Dose Immunomodulatory Studies of the active extracts  

B1: Cicer microphyllum (I^3M/38/A001) whole plant alcoholic extract  

A) IMMUNOMODULATORY STUDIES IN NORMAL ANIMALS  
1. In-vitro Studies  
1.1 Lymphocyte Proliferation Assay  
1.2 Phagocytic response  
1.3 Cytokine expression in murine PBMCs in-vitro  
2. In-vivo Studies
2.1 Cellular immune response (Delayed type hypersensitivity response)
2.2 Effect of I3M/38/A001 on T-cell surface markers in SRBCs immunized mice
2.3 Effect of I3M/38/A001 on intracellular cytokines.
2.4 Flow cytometric analysis of co stimulatory molecules.

B) IMMUNOMODULATORY STUDIES IN CYCLOSPORINE-A INDUCED IMMUNE-SUPPRESSED ANIMALS
1. Flowcytometric analysis of T cell surface markers
2. Flowcytometric analysis of Th1 / Th2 cytokines in whole blood
3. Vascular permeability in cyclosporine A induced immune suppressed mice
4. Skin allograft rejection

A) IMMUNOMODULATORY STUDIES IN NORMAL ANIMALS
1. In-vitro Studies
   1.1 Lymphocyte Proliferation Assay
   1.2 Phagocytic response
   1.3 Effect of TO-10 on cytokines expression in murine PBMCs in-vitro
2. In-vivo immunomodulatory studies in SRBCs immunized normal male
   2.1 Cellular immune response (Delayed type hypersensitivity response)
   2.2 Effect of TO-10 on T-cell surface markers in SRBCs immunized mice
   2.3 Effect of I3M/38/A001 on intracellular cytokines.
   2.4 Flowcytometric analysis of co stimulatory molecules.

B) IMMUNOMODULATORY STUDIES IN CYCLOSPORINE-A INDUCED IMMUNE-SUPPRESSED ANIMALS
1. Flowcytometric analysis of T cell surface markers
2. Flowcytometric analysis of Th1 / Th2 cytokines in whole blood
3. Vascular permeability in cyclosporine A induced immune suppressed mice
4. Skin allograft rejection

SECTION-C: Antistress evaluation of Cicer microphyllum (I3M/38/A001) and Taraxacum officinale (TO-10)
A) Cicer microphyllum (I\textsuperscript{3}M/38/A001)

1. Chronic restraint stress
   1.1 Effect of I\textsuperscript{3}M/38/A001 on T and B cell surface markers
   1.2 Effect of I\textsuperscript{3}M/38/A001 on CD244\textsuperscript{+} NK cells population
   1.3 Intracellular cytokine estimation.
   1.4 Estimation of polymorphonuclear leucocytes count.
   1.5 Corticosterone assay

2. SRBCs immunization in chronically restraint stressed mice
   2.1 Effect of I\textsuperscript{3}M/38/A001 on T cell surface markers in SRBC immunized chronically stressed mice.
   2.2 Effect of I\textsuperscript{3}M/38/A001 on CD4 specific IL-2, IFN-gamma and IL-4 levels.
   2.3 Effect of I\textsuperscript{3}M/38/A001 on whole blood IL-12 and IL-10 levels in chronically stressed mice.
   2.4 Extracellular cytokines estimation.
   2.5 Flowcytometric analysis of co stimulatory molecules.
   2.6 Delayed Type hypersensitivity response in stressed animals.
   2.7 Corticosterone assay
   2.8 Body and organ weights ratio

B) Taraxacum officinale (TO-10)

1. Chronic restraint stress
   1.1 Effect of TO-10 on T and B cell surface markers
   1.2 Effect of TO-10 on CD244\textsuperscript{+} NK cells population
   1.3 Intracellular cytokine estimation.
   1.4 Estimation of polymorphonuclear leucocytes count.
   1.5 Corticosterone assay

2. SRBCs immunization in chronically restraint stressed mice
   2.1 Effect of TO-10 on T cell surface markers in SRBC immunized chronically stressed mice.
   2.2 Effect of TO-10 on CD4 specific IL-2, IFN-gamma and IL-4 levels.
   2.3 Effect of TO-10 on whole blood IL-12 and IL-10 levels in chronically stressed mice.
2.4 Extracellular cytokines estimation.

2.5 Flowcytometric analysis of co stimulatory molecules.

2.6 Delayed Type hypersensitivity response in stressed animals.

2.7 Corticosterone assay

2.8 Body and organ weights ratio

SECTION-D: Fractionation of I⁢M/38/A001 and TO-10

SECTION-E: Detailed antistress investigation of chloroform fraction (C.F).

SECTION-F: Identification of the Active molecule

A) PRIMARY SCREENING

1.1 Effect of CA and CGA on IL-2 expression in murine PBMCs

B) IMMUNOMODULATORY SCREENING

Phase I

1.1 Effect of CA on Total T and B-cell surface markers in SRBCs immunized mice

1.2 Effect of CA on IFN-gamma (Th1) and IL-4 (Th2) expression in SRBCs immunized mice.

Phase II

1.1 Effect of CA on CD4⁺/CD8⁺ T cells population in peripheral blood in SRBCs immunized mice

1.2 Effect of CA on CD4 specific IFN-gamma, IL-2 (Th1) and IL-4 (Th2) expression in peripheral blood

B) EVALUATION OF ANTISTRESS ACTIVITY

1. Chronic restraint stress

1.1 Effect of CA on T and B cell surface markers

1.2 Effect of I⁢M/38/A001 on CD244⁺ NK cells population

1.3 Intracellular cytokine estimation.

1.4 Estimation of polymorphonuclear leucocytes count.

1.5 Corticosterone assay

2. SRBCs immunization in chronically restraint stressed mice

2.1 Effect of CA on CON-A and LPS stimulated splenocytes (ex-vivo).

2.2 Lymphocyte immunophenotyping

2.3 Effect of CA on Th1/Th2 cytokines in peripheral blood.
2.4 Extracellular cytokines estimation.
2.5 Effect of CA on the CD4+ CD25+ T cells.
2.6 Effect of CA on the expression CD28+ and CTLA-4 molecules.
2.7 Effect of CA on CD 80/86 expression
2.8 Corticosterone assay
2.9 Body and organ weights ratio

SECTION-G: Evaluation of the effect of ‘CA’ on chronic stress induced biochemical and behavioral alterations.
1. Chronic stress induced biochemical alteration
   1.1 Estimation of biochemical parameters in adrenal glands.
   1.2 Estimation of hepatic parameters.
   1.3 Estimation of biochemical parameters in serum
2. Chronic stress induced behavioral alteration
   2.1 Estimation of neurotransmitters in brain region

SECTION-H: Evaluation of the effect of ‘CA’ on chronic stress induced delayed wound healing.
1. Estimation of cytokines at the wound site

SECTION-1: Adaptogenic Studies
1. Anti fatigue effect
2. Swimming Endurance Test
3. Hypoxia Test

5. DISCUSSION
6. SUMMARY & CONCLUSION
7. REFERENCES
8. LIST OF PUBLICATIONS