TABLE OF CONTENT

LIST OF FIGURES xii-xiv
LIST OF TABLES Xv
LIST OF ABBREVIATIONS xvi
ABSTRACT 1-3
CHAPTER 1: INTRODUCTION 4-7
CHAPTER 2: REVIEW OF LITERATURE 8-50
  2.1 Carcinogenesis 8-9
    2.1.1 Initiation 8
    2.2.2 Promotion 8-9
    2.2.3 Progression 9
  2.2 Hallmarks of cancer 10
  2.3 World-wide cancer statistics 11-13
  2.4 Colon cancer 14-18
    2.4.1 Anatomy of colon 14
    2.4.2 Colon cancer statistics 14-15
    2.4.3 Risk factors 15-18
      2.4.3.1 Non-modifiable risks factors 15
      2.4.3.2 Modifiable risks factors 16-18
  2.5 Genetics of Colon Cancer 19-23
  2.6 Treatment 24-27
    2.6.1 Surgery 24
    2.6.2 Chemotherapy 24
    2.6.3 Radiation therapy 24
    2.6.4 Immunotherapy 25
2.6.5 Anti-angiogenesis
2.6.6. p53-Based Cancer Therapy
2.6.7 Targeting COX- inflammatory pathway
2.6.8 Apoptosis induction
2.6.9 Cytotoxics

2.7 Side effects associated with conventional therapies
2.7.1. Side-effects
2.7.2 Multiple drug resistance (MDR)

2.8 Mechanism of action of standard drug against colon cancer
2.8.1 5 FU (5 Fluouracil)
2.8.2 Mode of action
2.8.2.1 TS (Thymidylate Synthase) inhibition
2.8.2.2 RNA Misincorporation
2.8.2.3 Apoptosis and Cell Cycle Arrest

2.9 Mode of resistance developed by 5-FU
2.9.1 Cell cycle
2.9.2 Increase in TS levels
2.9.3 Anti-apoptosis

2.10 Emerging new anti-cancer therapies
2.10.1 Defining CAM therapies
2.10.2 Different forms of CAM therapies
2.10.2.1 Mind–body medicine
2.10.2.2. Alternative medical systems
2.10.2.3. Lifestyle and disease prevention
2.10.2.4 Biologically-based therapies
2.10.2.5 Manipulative and body-based systems
2.10.2.6. Biofield
2.10.2.7. Bioelectromagnetics

2.11. Trends of CAM therapies in India

2.12. Targeting Apoptosis and cell cycle in Colon cancer
2.12.1 Activation of programmed cell death (PCD) pathways
2.12.1.1 Extrinsic/ cell death receptor pathway 38
2.12.1.2 Intrinsic pathways 38-39
2.12.1.3 Role of plants in apoptotic induction 39-40
2.12.2 Cell cycle Arrest 40-44
2.12.2.1 Cell cycle machinery 41
2.12.2.2 Cell cycle regulation 41-43
2.12.2.3 Role of plants in cell cycle arrest 43-44

2.13 CAM sources used in the present study 45-50
2.13.1 *Triticum aestivum* (wheatgrass) 45-46
2.13.2 *Achyranthes Aspera* 46-47
2.13.3 Mushrooms 47-49
2.13.4 Homeopathy 49-50

CHAPTER 3 METHODOLOGY 51-74

3.1. Collection of Test Samples 51
3.1.1. *Triticum aestivum* 51
3.1.2. *Achyranthes aspera* 51
3.1.3. Mushrooms 51
3.1.4. Homeopathic remedy 51

3.2. Cell Lines 52

3.3. Preparation of Test Samples 52-53
3.3.1. Preparation of wheatgrass extracts 52-53
3.3.2. Preparation of *Achyranthes aspera* (AA) roots extracts 53
3.3.3. Preparation of mushroom extracts 53
3.3.4. Homeopathic drug 53

3.4. Study Design 54

3.5. Estimation of Total Phenolic Content (TPC) 55

3.6. Determination of Free Radical Scavenging by DPPH Assay 56

3.7. Determination of Free Radical Scavenging by FRAP Assay 57

3.8. Cell cytotoxicity by Trypan Blue Exclusion Assay 57-58
3.9. Cell Cytotoxicity by MTT Assay

3.10. Cell Growth Kinetics

3.11. *In Vitro* Wound Scratch Assay

3.12. Clonogenic Survival Assay

3.13. Anti-proliferative Assay

3.14. Detection of Apoptosis

3.14.1. Hoechst 33258 staining

3.14.2. Acridine Orange staining

3.14.3. Annexin V/Propidium Iodide staining

3.14.4. Qualitative assessment of DNA fragmentation by agarose gel electrophoresis

3.14.5. Quantitative assessment of DNA fragmentation by Diphenylamine (DPA) method

3.15. Gene Expression Studies

3.15.1. RNA isolation

3.15.2. cDNA synthesis and semi-quantitative PCR

3.16. Cell Cycle Analysis by Flow Cytometry

3.17. Statistical Analysis

CHAPTER – 4  RESULTS

4.1 Total phenolic content

4.2 *In vitro* antioxidant activity

4.3 Effect of CAM sources on colon cancer COLO-205 cells

4.3.1 Cell cytotoxicity by trypan blue exclusion assay

4.3.2 Cell cytotoxicity by MTT assay

4.3.3 Effect on growth

4.3.4 Anti-proliferative assay by trypan blue viability method

4.3.5 Effect of CAM sources on migration

4.3.6 Clonogenic survival assay

4.3.7 Apoptosis inductivity

4.3.7.1 Hoechst Staining

4.3.7.2 Acridine Orange staining
4.3.7.3 Annexin V/PI staining  
4.3.7.4 Qualitative assessment of DNA fragmentation by agarose gel electrophoresis  
4.3.7.5 Quantitative assessment of DNA fragmentation by DPA method  
4.3.8 Apoptosis related studies.  
4.3.9 Cell cycle analysis  
4.3.10 mRNA expression studies for cell cycle related genes.  
4.4 Effect of CAM sources on non-cancerous NRK-52E cells  
4.4.1 Evaluation of cytotoxicity by various CAM sources on NRK-52E cells.  
4.4.2 Assessment of morphological variations of NRK-52E cells  
4.4.3 Effect of CAM source and 5-FU on NRK-52E cell proliferation  
4.4.4 Apoptosis detection assay  
4.4.4.1 Acridine Orange (AO) staining  
4.4.4.2 DNA Fragmentation Assay  
4.5 Effect of cocktail mix on colon cancer COLO-205 cells  
4.5.1 Cell cytotoxicity by trypan blue exclusion assay  
4.5.2 Cell cytotoxicity by MTT assay  
4.5.3 Effect on growth  
4.5.4 Anti-proliferation effects  
4.5.5 Detection of apoptosis  
4.5.6 mRNA expression studies of apoptosis  
4.5.7 Cell cycle analysis  
4.5.8 mRNA expression of cell cycle related genes  
4.6 Effect of cocktail mix on normal NRK-52E cells  
4.6.1 Cell cytotoxicity  
4.6.2 Effect on cell growth  
4.6.3 Anti-proliferative Assay  
4.6.4 Detection of apoptosis  

CHAPTER 5: DISCUSSION
CHAPTER 6: CONCLUSION

LIST OF PUBLICATIONS

REFERENCES