CHAPTER – VIII
SUMMARY & CONCLUSION
SUMMARY AND CONCLUSIONS:

For investigations on leukaemia with a view to successful approach towards Immunotherapy, an experimental mouse model was produced. Young mice ageing 7-10 days has been injected (i.p) with N-N' ethylnitrosourea (ENU) at a dose rate of 80 mg/kg body weight and they are observed for 6-8 months time. Leukaemia induction was investigated through peripheral blood smear study by tail vein puncture. The leishman stained smear showed a mixed type of leukaemia, predominantly lymphoblastoid in nature. Induction of leukaemia by ENU, which becomes very toxic for the animals and around 15% of the animals died due to acute toxicity, developed leukaemia and survived 100-150 days of total life span, the rest 25% showed secondary infections with loss of hair, foot and mouth infections, anorexia, bleeding episodes, diarrhea and shorter life span, survived 45-50 days of total life span.

To combat the leukaeamic conditions which is induced by ENU into the mice, immuno-therapeutic strategy has been taken in which, combined immunopotentiators are introduced as a therapeutic agent. In this context, for the modulation of the immuno-effector cells balanced Biological Response Modifiers have been chosen in which Interleukin-2 (IL-2), Interferon-γ (IFN-γ) are taken as a specific and sheep erythocyte (SRBC) as a non-specific modulators. Administration of IL-2, IFN-γ and SRBC either alone or in combinations enhanced rate of survival against leukaemic conditions. Besides the rate of survival, it also potentiate the immunoeffecter cells that can be determined through their activities. After the application of IFN-γ or SRBC, it has been found that E'-rosetting capacity is increased but application of combination of SRBC, IL-2 and IFN-γ promote the formation of E' rosette in controlled way. On
the other hand cytolytic capacity of T-lymphocyte is increased when treated with IL-2 or SRBC alone or in combination, but IFN-γ does not show any beneficial significance excepting in combination with IL-2 and SRBC. Studies conducted with PMNs also revealed a significant increase in plagocytic burst against the tumour targets when treated with SRBC or IFN-γ. But maximum results obtained after the treatment of SRBC + IL-2 and IFN-γ in combination. An increased antigen presenting capacity by macrophages and adherence capacity has been found to be significantly elevated following SRBC and IL-2 treatment. Functional activities of macrophages are found to be beneficial after the treatment of SRBC, IL-2 and IFN-γ in combination. In reality a combination of specific or non-specific BRM therapy can produce a greater therapeutic index.

Protein tyrosine kinase is a type of second messenger molecule which regulates the genetic information at molecular level. Its activity has been measured in the streptavidin coated microtiter plates containing biotin labelled substrate peptide and an antiphosphatroyosine enzyme activity. Results showed that IL-2 acts towards the upregulation but IFN-γ contributes the negative or down regulatory aspects of the system concerned. In case of lymphocytes, protein tyrosine Kinase (PTK) activity is suppressed in leukaemic condition, whereas, in neutrophil in this condition PTK activity is increased. So, molecular basis of BRMs activity can be indicated through the knowledge about the performance of such signaling molecule i.e. protein tyrosin kinase activity. Our attempts to study the effects of BRMs through this works provided satisfaction and leaves ample scope of further research.

In the present context, after the investigation of BRMs, surface ultrastrucual studies have been considered to be very interesting with
respect to effector target interactions with or without administration of Biological Response Modifiers (BRMs). These included the serial investigation of surface ultrastructural characters of lymphocytes, neutrophil and macrophages under scanning electron microscope (SEM). In leukaemic conditions, lymphocytes of different categories, neutrophil, macrophages have been found to exhibit malformation of surface ultrastructure including ruffled membrane, blebbing and folded invaginations with necrotic foci. But after the application of BRMs like specific IL-2 or IFN- or non-specific SRBC either alone or in combination showed an active functional approaches of cellular interaction like europod formation, blebbing and genesis of excess microvilli. The above investigations provide valuable informations regarding surface ultrastructural behaviour of these cells in the leukaemic conditions and under the influence of BRMs as immunopotentiators.

The principle aim of the thesis work have satisfactory fulfillment in the context of newer therapeutic approaches in haematological malignancy in experimental animals. The conventional therapeutic approaches having immuno-suppressing characters awaited a suitable immunological adjunct since long. Biological Response Modifiers, including IL-2, IFN-γ and SRBC provided an able support to the immune system with a conquerent increased survival rate compared to the counter parts. The obvious mechanisms have been directed towards immuno-potentiation and increased cyto-toxicity against the tumour targets. Thus the cytokines with antitumour property and non-specific corpuscular antigen of SRBC indicated a new approach for anti leukaemic immunotherapy as revealed in experimental model. Applications in human cases also indicated a similar protection.