CHAPTER X

SUMMARY AND CONCLUSION
In modern times, there is an increase in the search of potential drugs that are capable of modifying immune responses with less serious side effects. Scientists view the herbal drugs with interest as they are generally available in bulky crude forms and are freshly made into concoction for oral administration according to prescription. Despite this, the elucidation of immunostimulatory properties of medicinal plants, lack of adverse effects and oral efficacy suggest that they may be useful as adjuvants in cancer therapies or in AIDS.

The use of plants as a source of immunomodulatory material is in a developing stage. They have been known to stimulate haemopoietic system and by the maturation of the immune cells. Some of the plants with known immunomodulatory activity are *Viscum album* which is being used in the cancer therapy (5), *Panax ginseng* (30), *Phyllanthus emblica* (31), *Tinospora cordifolia* (6), *Asparagus racemosus* etc. (20).

*Withania somnifera* popularly known as Aswagandha belonging to the family Solanaceae is used in several indigenous drug preparations for maintaining the general health and ojas (30). Withania was found to possess adaptogenic, antitumour (131,132) and anti-inflammatory activities (37). The major components present in Withania was found to be an essential oil – ipurinol, a crystalline alcohol- Withanol, hentria contane, phytosteroids, fatty acids and alkaloids – Withanolides (133). An important group of steroids, Withanolides, possess antitumour and antibacterial activities.

The results of the present study indicate that Withania extract and Withanolide D treatment
could significantly enhance total WBC count, bone marrow cellularity and alpha esterase positive cells in normal mice. The maximum count was observed 6 days after the initiation of drug treatment. There was no significant change in the haemoglobin level but the body weight of these animals was found to be increased after drug administration.

Administration of Withania extract and Withanolide D were found to activate humoral immune responses in mice. The weight of lymphoid organs such as spleen and thymus were found to be enhanced in Withania treated animals. There was an increased secretion of antibodies into circulation as well as antibody producing cells in the spleen against sheep red blood cells in mice treated with Withania extract. Maximum antibody titre was obtained in Withania treated group on 12th day and the titre retained up to 27th day, indicating that the immunological activity was sustained for several days. Maximum number of plaque forming cells were obtained very early in the Withania treated group. There was an increased production of immunoglobulins such as IgG and IgA by Withania treatment indicating its role in humoral immune responses in mice.

Withania extract was found to activate the cellular immune responses in mice. Administration of Withania extract could significantly enhance the phagocytic activity of peritoneal macrophages and inhibited delayed type hypersensitivity reaction in mice. Withania administration could significantly enhance the cytotoxic T lymphocyte production as it is evidenced by the increase in life span of thymoma bearing animals. Administration of Withania extract activated non specific immune cells such as natural killer cells (NK) and macrophages. NK cell activity was found to be enhanced in Withania and
Withanolide D treated normal and tumour bearing animals much earlier than the controls. Withania was found to activate and enhance antibody dependent cellular cytotoxicity (ADCC) and complement mediated cytotoxicity (ACC) in normal and tumour bearing animals and maximum lysis occurred much earlier compared to untreated controls. Administration of Withania extract and Withanolide D could significantly enhance the lymphocyte proliferation along with the mitogens such as PHA; Con - A, PWM and LPS. LPS could stimulate the Withania treated splenocytes six times more than the normal. Bone marrow cells from Withania treated animals were found to proliferate significantly. Withania somnifera and Withanolide D administration could significantly increase the bone marrow and thymocyte proliferation. Both PHA and Con - A mitogens could stimulate the Withania treated bone marrow and thymocyte proliferation twice greater than the normal. This shows that Withania could stimulate B and T lymphocyte proliferation in mice. These results indicate that the immunomodulation produced by Withania extract is due to the combined action of humoral and cell mediated immune responses in mice.

Immunosuppression is the major drawback in cancer chemotherapeutic practises. Cyclophosphamide (CTX) is an alkylating agent widely used in cancer chemotherapy. CTX administration causes severe toxic side effects such as nausea, vomiting, mucosal ulceration, dizziness out of which immunosuppression is the major one. Withania could significantly reduce the myelosuppression induced by chemotherapy. There was a significant increase in the WBC count and bone marrow cellularity within 48 h. after the completion of cyclophosphamide administration in Withania treated animals compared to controls. There was a significant enhancement in the body weight of mice treated with
Withania extract compared to CTX alone treated group. Administration of Withania extract along with CTX was shown to enhance the relative organ weight of spleen and thymus of mice compared to that of CTX alone treated group. The intestinal villi architecture of mice treated with CTX alone treated group showed complete distorsion and necrosis of mucous cells whereas CTX in the presence of Withania treated group showed normal architecture of intestinal villi indicating that Withania could reverse the intestinal toxicity induced by CTX.

Withania treatment enhanced the cytokine level in normal and CTX treated mice. Cytokines such as IFN-γ, IL-2 and GM-CSF were significantly increased in Withania treated mice while TNF-α was found to be lowered in Withania treated animals.

Another severe side effect of CTX administration is urotoxicity. Treatment with Withania extract could significantly alter the severe urotoxicity induced by cyclophosphamide treatment. Morphological appearance of bladder of CTX and Withania treated group was normal compared to CTX alone which was dark in colour and severely inflamed. Administration of Withania extract along with CTX could normalize the bladder pathology. Analysis of bladder of CTX alone treated group showed necrosis of cells and giant cells in epithelium and numerous acute and chronic inflammatory cells were present. But CTX along with the Withania showed no necrosis of cells and it looked like a normal bladder. Proteins and other metabolites accumulated in the urinary bladder cause oedema formation and severe damages in CTX treated group whereas CTX and Withania treated group showed no oedema formation or severe damages. Treatment with Withania extract along with CTX showed nor-
normal values of total protein, urea nitrogen in both serum and urine whereas CTX alone treated group showed drastically elevated levels of the above parameters. The lowered GSH content in liver and bladder in CTX alone treated group was normalized in Withania treated group. Morphological, histopathological and biochemical analysis showed that Withania could alleviate the severe urotoxicity induced by CTX treatment confirming the use of Withania in reducing the toxic side effects of chemotherapy.

*Withania somnifera* administration could significantly reverse the radiation induced toxicity. The decreased total WBC count, bone marrow cellularity and alpha esterase positive cells by radiation treatment were significantly elevated by Withania treatment. Withania could normalize the ratio of normochromatic to polychromatic erythrocytes in the bone marrow of radiation treated mice indicating that Withania could stimulate the stem cell proliferation. The number of spleen nodular colonies was increased by Withania treatment, in normal mice and in radiation treated animals indicating that Withania could stimulate colony forming unit derived from bone marrow cells.

Administration of *Withania somnifera* extract and its component Withaferin A could significantly inhibit the growth of the solid tumour induced by Daltons lymphoma ascites cells and ascites tumour induced by Ehrlich Ascites cells. Withania could synergistically reduce the tumour volume in the presence of radiation, cyclophosphamide and hyperthermia and the combined action of these modalities produced significant reduction in tumour volume. Hyperthermia along with Withania could stimulate the immune system and enhanced the level of myeloperoxidase level in mice with solid tumour
induced by DLA cells. These studies indicate that Withania could be used as a response modifier of other treatment modalities such as radiotherapy, chemotherapy and thermotherapy.

Administration of Withania extract could significantly inhibit the Dimethyl benzanthracene (DMBA) and croton oil induced papilloma as well as methyl cholanthrene induced fibrosarcoma very effectively both in terms of incidence of tumour and survival of animals which indicates its role in anticarcinogenic activity. There was a significant enhancement in the level of enzymes such as GST, Catalase, Glutathione peroxidase and increased GSH levels while inhibited lipid peroxides in Withania treated group compared to control tumour bearing animals. Mechanism of action of Withania extract in the inhibition of chemically induced carcinogenesis is due to its antioxidant and immunomodulatory activity.

Immunomodulatory activity of Withania is due to the combined action of humoral and cell mediated immune responses in mice. Withanolide D administration showed a significant elevation in all the immunological parameters indicating that the major activity of Withania is due to the presence of Withanolide D. Withaferin A treatment did not show any significant increase in immunological parameters but it was found to be a potent antitumour agent indicating that antitumour activity of Withania is mainly due to the presence of Withaferin A.

These results indicated that Withania could act as a nontoxic immunomodulator which possesses anticancer, chemoprotective and radioprotective properties. Use of *Withania somnifera* as an adjuvant during cancer chemotherapy and radiotherapy is highly recommended.