Chapter VI: Summary
6.1: Summary

✓ The present research work firstly involves immunostimulant property of Levamisole Hydrochloride in which pretreatment of Levamisole Hydrochloride is given to animals and then immunosuppressant is given after pretreatment, which can determine the immunostimulant ability of Levamisole Hydrochloride.

✓ The immunity level was accessed by different in vivo models like cyclophosphamide induce myelosuppression and neutrophil adhesion test which involves hematological determination of blood samples while other in vivo models like heamagglutination titre and delayed type hypersensitivity reaction which involves interaction with antigen (Sheep red blood cells).

✓ A nanoparticulate formulation was prepared by ionic gelation method and optimized for different properties.

✓ A three level three factor design of experiment was developed for optimization of nanoparticles. Optimization of nanoparticle was done by average particle size (nm), zeta potential (ζ) (mv) and percentage entrapment. Scale up of optimized formulation was done and characterized for morphology, drug release characteristic, infrared spectroscopy and thermal analysis.

✓ Stability study was also carried out at 25±2°C/60±5% RH and 4°C refrigerated condition in amber color bottle.

✓ Nanoparticles were accessed for their uptake property by Peyer’s patch (collection of lymph node) in which Chitosan was tagged with FITC and then this tagged Chitosan was used for nanoparticle preparation. The tagged nanoparticles were studied for uptake ability on caco-2 cell lines by fluorescence microscopy.

✓ The nanoparticle’s immunostimulant activity of Levamisole nanoparticles was compared with that of drug solution using same in vivo models i.e. cyclophosphamide induced myelosuppression; neutrophil adhesion test; delayed type hypersensitivity reaction model and heamagglutination assay titre and was found significant.
6.2: Conclusion

- Minimum 30 days pretreatment is able to maintain immunity against immunosuppression as shown from pharmacological studies.

- It was found that the negative charge generating agent, pH adjusting agent, pH of Chitosan solution, Chitosan concentration, pH of Levamisole Hydrochloride solution, Chitosan/STPP ratio and Levamisole Hydrochloride’s concentration are having impact on optimization parameter like average particle size, zeta potential, percentage drug entrapment, morphology of nanoparticles.

- 10% ammonia solution; pH 4.0±0.2 and 2 mg/ml were selected for pH adjusting agent; Chitosan solution pH and Chitosan concentration respectively.

- From the ANOVA table, it was concluded that non-significant parameters can’t be neglected for any responses.

- It was observed that at lower level of Chitosan/STPP; pH of Levamisole dissolved STPP solution and Levamisole concentration, highest percentage entrapment (38±3.2 %) was seen with good particle size (135.8±12.4 nm) and zeta potential (31.50±1.3 mv).

- No much change in formulation characteristics like percentage entrapment, zeta potential and particle size even after scale up.

- Initial burst release of Levamisole may be due to swelling of nanoparticles in acidic condition and/or adsorption of some of the drug on nanoparticles matrix. Overall nanoparticles shows sustained release for 24 hr. The drug release kinetics shows mix pattern of first order and Hixson-Crowell model.

- From TEM images, it was observed that nanoparticles are spherical in shape and solid in structure.

- From DSC analysis it can be concluded that, the characteristic peak of Levamisole and Chitosan-STPP nanoparticles were absent in the Levamisole loaded nanoparticles indicating the Levamisole is dispersed molecularly in the Chitosan-STPP complex.

- From FTIR study it can be concluded that Levamisole interacts strongly with Chitosan-STPP nanoparticle matrix causing change in the peak of Levamisole alone or Chitosan-STPP nanoparticles alone.

- Nanoparticles are found stable at 4-8°C for 12 months.
Fluorescence microscopic images confirm uptake of FITC labeled nanoparticles by caco-2 cell lines.

The Levamisole nanoparticles have also shown statistically significant immunostimulant activity compared to Levamisole solution alone.

It should be noted that role of payer’s is to pick, process and present antigen to help generate specific immune response, depending on the nature of the particular antigen. It is proved that Levamisole is effective in inducing antibody production by increasing T and B lymphocyte responsiveness, probably through facilitating a greater contact with the antigen overtime. (66; 58)

Thus levamisole can act as non-specific immunostimulant. The levamisole loaded nanoparticles are also able to be uptake by caco-2 cell line, which confirms their ability to be uptake by Peyer’s patches.

Thus the Levamisole nanoparticles, which are targeted to Peyer’s patches trigger’s the antibody production by facilitating a greater contact with the antigen, acts as nonspecific immunostimulant.

6.3: Future prospects

By the application of nanotechnology, not only the dose and toxicity of drug can be decreased but also the site specificity can be increased.

Thus the future scope of the present research work mainly include targeting of various agents to Peyer’s patch and immuno-maintenance by giving nanoparticles for pretreatment which will be non-specific in nature and can be useful in many infectious diseases caused due to immunosuppression.

Thus the formulation will be having better applicability compared to vaccines as it is non-specific in nature and can be useful in all the immunosuppressed condition caused diseases