Immunostimulatory agents are used to stimulate the immune response of an organism against the invading antigens. Innate or nonspecific immunity is an essential first-line of defense against microbial pathogens by activation of phagocytic cells, such as macrophages and neutrophils. Humoral or specific immunity is produced by the formation of specific lymphocytes which producing antibody. Thus, enhancement of nonspecifically or innate immunity and specific or humoral immune response could increase defense against various diseases. Initial interest in the immunomodulatory properties of polysaccharides was raised after experiments which showed that a crude yeast cell preparation stimulated macrophages via activation of the complement system. During the past three decades, a number of bioactive polysaccharides and polysaccharide–protein complexes have been isolated from mushrooms, fungi, yeast, algae, lichens and plants which have attracted significant attention because of their immunostimulatory effects. Moreover, most plant-derived polysaccharides are relatively nontoxic and do not cause severe side effects, compare to the immunomodulatory bacterial polysaccharides and other synthetic compounds. The plant polysaccharides have been also reported for producing immunosynergistic effect with combination to natural and synthetic drugs. The polysaccharide fraction isolated from different plants and purified by cellulose, Sephadex have shown synergistic effect in combination with other drugs (e.g. Panax ginseng synergistic immunostimulatory effect with interferon-\(\text{\textgamma}\), 5-fluorouracil).

Levamisole, an anthelmintic drug can restore the depressed immune function through stimulating antibody formation and enhance T-cell response by stimulating T-cell activation and proliferation. The drug can also enhance the phagocytosis of phagocytic cell,
lymphocyte proliferation, number of plaque-forming cells and NK cell activity. Levamisole in combination with *Asparagus racemosus*, *Sida cordifolia* showed significant synergistic immunostimulation effect in bird.

Natural polysaccharides are recently getting consideration as biopolymers as they are economical, easily available, non toxic, readily modified, biodegradable and biocompatible. They are having extensive applications in pharmaceutical and food industry because of their diversity in structure and properties. Natural polysaccharide also used as bioadhesive excipients in different formulations such as oral mucoadhesive tablets and buccal tablet.

Gum isolated from the fruit pulp of *Aegle marmelos* was successfully used as tablet binder and excipients. However, information about physico-chemical characterization of purified polysaccharide, assessment of its potentiality as mucoadhesive excipient and combination effect of the purified polysaccharide isolated from the fruit pulp of *Aegle marmelos* with Levamisole in relation to immunological change in experimental animal have not been studied thoroughly.

So the aim of the present study was (1) to isolate and purify the polysaccharides from fruit pulp of *Aegle marmelos* and investigate their physico-chemical properties for the suitability of use as pharmaceutical excipients (2) to formulate and evaluate the mucoadhesive property of the tablet formulation by using both *in vitro* and *in vivo* methods where purified polysaccharide is used as excipient and levamisole as a model drug. (3) to
evaluate the immune synergistic potentiality of the polysaccharide in combination with levamisole.

Chapter 1 of the thesis deals with the background of the investigation and a brief introduction to immune system, immunotherapy and natural immunotherapeutic agent where special emphasis on immunostimulatory and synergistic immunostimulatory activity of polysaccharides are given. The next part of this chapter contains mucoadhesive drug delivery system with its different aspects. The role of natural mucoadhesive polysaccharide as excipients in drug delivery system, their advantages and recent application were also discussed. Further, an introduction to Aegle marmelos and the drug profile of Levamisole have been presented.

Chapter 2 represents some reports on Immunostimulatory activities of plant extract and natural polysaccharide along with their immunosynergistic potential. Various research evidences on natural gums, mucilage and polysaccharides isolated from natural sources, their isolations, purifications and characterization procedure has been presented. The current updated status of polysaccharides used as pharmaceutical excipients and mucoadhesive drug delivery has been elaborated in the form of comprehensive review of literature. Different parts of Aegle marmelos has been discussed with their traditional use and in modern system of medicine. Usage of Aegle marmelos gum as a pharmaceutical excipient also exists in the chapter.

Chapter 3 deals with the major aim, objective and plan of the work. This includes a brief description of the approaches used to achieve the objectives.
Chapter 4 deals with the evaluation of *Aegle marmelos* as pharmaceutical excipients. Polysaccharides were isolated and purified by gel filtration chromatographic method.

To characterize the polysaccharides by assessing particle size distribution in polysaccharide powder, flow properties such as bulk density, tapped density, compressibility index, Haunser ratio and angle of repose were studied. The microbial quality test was conducted to detect the load of different species of bacteria, yeast and moulds. Ash values, moisture content, solubility and pH were determined for the polysaccharides to evaluate their purity and suitability as excipients for pharmaceutical formulations. Elemental analysis, scanning electron microscopy (SEM), X-ray diffraction crystallography (XRD), differential scanning calorimetry (DSC), Fourier-transform infrared spectroscopy (FT-IR) techniques were used to characterize the polysaccharides.

The experimental results of the derived polysaccharide *Aegle marmelos* (AMPS) revealed that the polysaccharide was non-starch in nature. The characterization studies gave promising results of AMPS indicating good flow, compressibility and high swelling index confirming its suitability as an excipient. Results further confirmed that AMPS is a suitable natural polysaccharide and carrier to improve the solubility and dissolution rates of poorly water soluble drugs. Elemental analysis of the polysaccharide indicated the contents of carbon, hydrogen and nitrogen to be 27.20, 4.07 and 0.62 (w/w %) respectively. SEM analysis suggested that the polysaccharide had irregular particle size, mostly seen in aggregates and fibrous in nature. The samples had peaks at approximately 28°, 32°, 42° and 45° 20 degrees in the XRD pattern, which indicated both crystalline and amorphous
structure. The IR spectrum showed Peaks at 3246.85 cm\(^{-1}\) due to -OH stretching of primary alcohol. The absorption peaks at 2976 cm\(^{-1}\) and 2889 cm\(^{-1}\) are indicative of –CH stretching vibration of methyl group. The absence of significant aromatic stretches in the 1739 cm\(^{-1}\) region and the weakness of the stretches imply that there is modest amount of cross linking by peptides. The bands at 1607 cm\(^{-1}\) is characteristic of C=O of aldehyde. Peak at 1374 cm\(^{-1}\) is due to symmetrical deformation of -CH\(_2\) and C-OH group. Weak bond at 769.38 cm\(^{-1}\) due to the r contribute to the ring stretching and ring deformation of \(\alpha\)-D-(1-4) & \(\alpha\)-D-(1-6) linkage. Analytical data on AMPS indicated that the major neutral sugars were \(\alpha\)-D-glucose, \(\beta\)-D-glucose, rhamnose and D- Glucuronic acid. The results obtained in this study were established for the first time regarding the physicochemical & structural characteristics of the polysaccharide from the fruit of Aegle marmelos. The purified polysaccharide with such properties has therefore been used as a polymer in novel drug delivery systems to prolong drug release. The high thermal stability of the polysaccharide makes it suitable as an excipient even under conditions of high thermal stress. The relative abundance and easy availability of AMPS may serve as an alternative to present available pharmaceutical excipients.

Chapter 5 deals with Toxicity test of purified polysaccharide extracted from Aegle marmelos for discerning the compilations arising from the therapeutic use and to evaluate its safety potential. A special attention has paid during the period of the toxicity tests for care of the Swiss albino mice of female sex weighing 20-25gms as per OECD guideline. The animals were acclimatized to laboratory conditions and four doses were selected (5mg/kg, 50mg/kg, 300mg/kg and 2000mg/ kg) as per guideline.
It was observed that the animals fed with the *Aegle marmelos* polysaccharide were found to be healthy and their mortality and morbidity (convulsions, tremors, and grip strength and pupil dilatation) at an interval of 12 h for 14 days are normal. No unusual changes in behavior or in locomotors activity, ataxia, and signs of toxicity were observed during the 14 days period.

On the basis of the acute toxicity study it was concluded that *Aegle marmelos* polysaccharide was nontoxic in female Swiss albino mice (which is most sensitive to the toxic effect of drug) and could be used in further study in Swiss albino mice within the dose of 2000mg/kg body weight. It can also be predicted that *Aegle marmelos* polysaccharide could be similarly safe in Newzeland rabbit in terms of toxicity as we found in female Swiss albino mice.

**Chapter 6** deals with the preparation and evaluation of mucoadhesive controlled release tablets by using *Aegle marmelos* polysaccharide. The tablets were formulated by wet granulation method after evaluating drug-excipient interaction studies. Levamisole was taken as a model drug to study the mucoadhesive property of the isolated purified polysaccharide AMPS. Nine different formulations were prepared (F10-F50) with different concentrations of polysaccharide. The tablets were evaluated for their physical properties like weight variation, hardness, friability and content uniformity. Dissolution study was conducted to characterize release mechanism from the matrix system and data were fitted to various kinetic models. The mucoadhesive property was evaluated different in *vitro*, ex *vivo* and in *vivo* models.
The drug-excipient interaction studies (FTIR and DSC) indicated no chemical interaction between levamisole and polysaccharides used in the experiment.

The results of all the formulations for the mucoadhesive tablets of AMPS showed good physical characteristics. Weight variation data of the prepared tablets indicated no significant difference in the weight of individual tablet from the average value. Hardness, friability and uniformity content of all the formulation were found within the official limits. In vitro release studies indicated sustained release of Levamisole extending the drug release up to 10 to 12 hrs for all the formulations. An increase in polymer concentration resulted in decrease in drug release due to increased viscosity causing thicker gel layer with longer diffusion path. The drug release from all the prepared tablets had shown mechanisms involving a combination of both diffusion and erosion.

In vitro mucoadhesive measured as detachment force in grams for the polymer and has shown successful result as compared to synthetic polymer as per literature. Ex vivo mucoadhesive strength and ex vivo residence time studies showed that formulation F40 batch showed satisfactory mucoadhesive properties. From the encouraging results of the in vitro dissolution study, mucoadhesive strength and mucoadhesive time data, the formulation was found to be promising and was further considered for in vivo imaging (Scintigraphy & X-ray studies) and in vivo bioavailability studies. The product adhered in the gastric mucosa for about 12 hrs.

There is a significant increase in the oral bioavailability of the levamisole from the AMPS tablet which was evident from the increased AUC (~2 times) than that of the
commercial formulation. There was no significant difference in $C_{\text{max}}$ values among LEVASOL 50 and AMPS mucoadhesive tablets F 40. The experimental formulations (AMPS tablet F 40) exhibited longer $T_{\text{max}}$, $t_{1/2}$ and MRT values than the commercial formulation, which indicated that experimental formulations followed sustained release pattern compared to marketed immediate release formulation.

Chapter 7 deals with the evaluation of immunostimulatory and synergistic immunostimulatory potential of the AMPS and AMPS in combination of Levamisole. The study was designed into four in-vivo animal models for the assessment of cellular and humoral immune responses. Carbon clearance test and neutrophil adhesion test for evaluation of cellular immunity and haemaglutination titer and Delayed type of hypersensitivity for humoral immune response. Since all the doses of AMPS in combination with levamisole showed remarkable augmentation in the phagocytic index it can be speculated that it might be due to increase in the activity of the reticuloendothelial system by prior treatment of animals with AMPS with Levamisole in combination which might be possessing some synergistic potential in activating reticuloendothelial system. Same types of activity were observed in case of neutrophil adhesion test. Levamisole is a known immunostimulant, but in combination with AMPS its activity on marinating polymorphoneuclear neutrophil has been remarkably increased. This increased in activity may be due to the synergistic effect in combination of Levamisole with polysaccharide. The polysaccharide itself also showed some immunostimulation activity which was increased in dose dependent manner. Haemaglutination titer value also increased when mice were treated with AMPS with levamisole. It indicates that the combination also
synergistically increased the humoral antibody response. Similar types of response in DTH also indicate that the synergistic potentiality of that combination on TH1 cell. Hence, we can conclude that polysaccharide isolated from the fruit pulp of *Aegle marmelos* in combination with Levamisole have synergistic immunotherapeutic potential and further studies for elucidation of the mechanism of action are recommended.

Results of the present study clearly indicated that mucoadhesive tablets of Levamisole with AMPS were successfully formulated and optimized. These systems were retained in the stomach for longer periods and released the drug in a sustained manner. Hence, the developed gastric mucoadhesive systems can provide a sustained release effect with good oral bioavailability for drugs like Levamisole that are having site specific absorption in the stomach or the upper parts of the small intestine.

Thus, the polysaccharides extracted from *Aegle marmelos* can be used as immune synergistic mucoadhesive polymers for drug Levamisole, which will be alternatives to the existing or synthetic products because of their superiority in terms of biocompatibility, non-toxicity, biodegradability, environmental friendly nature and low prices.