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Original Article

Immunostimulatory Activity of Chitosan Nanoparticles on Wistar Albino Rats

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ABSTRACT

Background: The active components of natural products provide a potential alternative to conventional immunotherapy for a variety of diseases conditions and become subject to scientific investigations currently worldwide. **Objective:** The purpose of this research was to investigate the immunostimulatory activity of the chitosan nanoparticle on Wistar albino rats. **Materials and Methods:** The present investigation was carried out on various groups of healthy adult rats. The assessment of immunomodulatory potential was carried out by neutrophil adhesion test, delayed-type hypersensitivity (DTH) response, haemagglutinating antibody (HA) titre, cyclophosphamide-induced myelosuppression and phagocytic activity were determined in various groups of animals. **Results:** The administration of chitosan nanoparticle at doses 300 mg/kg BW and 600 mg/kg BW but not at doses 150 mg/kg BW significantly increased in neutrophil adhesion fibers, haemagglutinating antibody titre values and potentiated the inhibited type hypersensitivity reaction induced by sheep red blood cells. Also, it had good response towards phagocytosis in carbon clearance assay and prevented myelosuppression of cyclophosphamide on rats. **Conclusion:** From these findings, it can be concluded that chitosan nanoparticle responsible for immunostimulatory activity and has therapeutic potential for the prevention of immune depressed conditions.

Key words: Chitosan Nanoparticle, Neutrophil adhesion, Delayed-Type Hypersensitivity, Haemagglutinating antibody, Myelosuppression, Phagocytosis.

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INTRODUCTION

Immunomodulatory using natural product can provide an alternative to conventional immunotherapy for a variety of diseases, especially when impaired immune responsiveness of the host's in under conditions as immunostimulatory or when a selective immunosuppressant has to be induced in a situation like autoimmune disorders and organ transplantation as immunosuppressor.[1,2] Immunostimulatory may be synthetic drugs or of natural product origin. Due to the severe side effects related to synthetic drugs, immunostimulation using natural product drugs can provide an alternative to conventional chemotherapy for a variety of diseases, especially when the host defense mechanism has to be activated under the conditions of an impaired immune response.[3] Natural product immunostimulant are easily affordable and are also less likely to cause side effects.

The active components of natural products provide a potential alternative to conventional immunotherapy for a variety of immunologic diseases and become subject to scientific investigations for many researcher. In this context, the development of medicinal natural product-based immunostimulatory has gained momentum in research studies directed toward design and discovery of immunostimulant drugs.[4,5] Medicinal natural products have long been used as immunostimulatory in traditional medicine, for the treatment of many immunological disorders. *Andrographis paniculata, Allium sativum, Cajanus indicus, Gymnema sylvestre, Asparagus racemosus, Piper longum* Linn, *Curcuma longa, Phyllanthus emblica* Linn, *Ocimum sanctum* Linn., *Tinospora cordifolia, Pinus merkusii* and chitosan are among the natural product claimed to possess potential immunostimulatory agent.[6-10]

Based on several studies, it is reported that chitosan, one of the natural product active compounds derived from the sea, has potent immunostimulatory properties.[9,10] Over the last three decades, there has been increasing number of publications on chitosan and its derivatives in the pharmaceutical industry. The chitosan is known to possess many biological activities such as antibacterial,[11,12] antioxidant,[13] matrix metalloproteinase (MMP) inhibition,[14] anti-diabetic,[15] anti-HIV,[16] anti-inflammatory activities,[17] drug delivery,[18] and immunoenhancing,[9,10] etc. Not

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only restricted to those activities, but also chitosan modification will enhance and open various ways to utilize chitosan.[19] The rationale for this is that chitosan modification will keep the original physiochemical and biochemical properties of chitosan and bring the new properties of the group introduced to them at the same time.[8,11,20]

The chitosan nanoparticle has received much attention from researchers which has been related to their potential application, especially in medicine.[21,22] Nanoparticle chitosan has gained growing interest due to its biodegradability, biocompatibility, high permeability, non-toxic property, cost-effectiveness and excellent film forming ability. Moreover, it has been recognized as mucoadhesivity chitosan and has the ability to enhance the penetration of large molecules across a mucosal surface.[23,24] However, the immunostimulatory effect of chitosan nanoparticles has seldom been reported elsewhere. Chitosan nanoparticles have an important role for the development of a new drug with improved biodistribution and increased specificity and sensitivity and reduced pharmacological toxicity. The aim of the present study is to a synthesis and evaluate of the chitosan nanoparticles for immunostimulatory applications.

MATERIALS AND METHODS

Experimental animals

Male Wistar albino rat weighing approximately 200-250 g (2.5-3 months) were purchased from Gadjah Mada University, Yogyakarta, Indonesia. They were housed in plastic cages with a temperature maintained at 26 ± 2 ° C and 12 hr alternates light and dark cycles. The rats were given *ad libitum* with drinking water and fed with a standard commercial rat. This study was conducted by the Ethical Clearance Committee for preclinical research, Institute of Tropical Disease, Airlangga University and obtained ethical clearance under No.109/ITD/8/2017.

Preparation of chitosan

The production of chitosan from crustacean shell consists of demineralization, deproteinization, and deacetylation.[11] The shells were demineralized by agitating continuously with 5% HCl at the ratio of 1:15 (w/v, shell to a solution) 36 hr at room temperature. The demineralized shells were treated with 5% NaOH solution at the ratio of shell to a solution of 1:10 (w/v) at 90-95°C for 6 hr. The deproteinized shells were washed and filtered with tap water until NaOH was removed completely, then dried overnight in an oven at 55-60°C. The shells were filtered and washed with tap water until became neutral. Then deacetylation of chitosan was carried out by hydrolyzing with 80% NaOH at the ratio of 1:20 (w/v, chitin to solvent) at 90-95°C for 5 hr. This product was washed with tap water until it became neutral and dried overnight at 55-60°C. In the preparation of chitosan solutions, 1.0% (w/v) chitosans were dispersed in a 1.0% (v/v) acetic acid solution.

Preparation of chitosan nanoparticles

In the present study, the chitosan nanoparticles were synthesized from the chitosan using sodium tripolyphosphate as a crosslinking agent by ionotropic gelation method.[11,22,23] Initially in order to create the homogeneous chitosan solution, about 1.5 g of chitosan dissolved in 200 ml of 2% acetic acid solution was kept under magnetic stirring process for about 20 min. Furthermore, to the above prepared chitosan solution, 0.8 g of sodium tripolyphosphate dissolved in 107 ml of conductivity water was added drop wise and stirred well for about 30 min to reach equilibrium. The emulsion of chitosan nanoparticles seem like milky colored emulsion, which was formed upon the ionic cross linking between the sodium tripolyphosphate and chitosan solution. After reaching equilibrium, The suspension was formed in above mentioned conditions. The nanoparticles were separated by centrifugation at 20,000 g and 14°C for 30 min, freeze-dried and stored at 5 ± 3°C.

Sheep red blood cells preparation

Fresh blood was collected from sheep sacrificed in the local slaughter house. Sheep red blood cells (SRBCs) were washed three times in large volumes of pyrogen free 0.9 % normal saline and adjusted to a concentration of 0.5×10^9 cells/ml for immunization and challenge.[24]

Neutrophil adhesion test

Immunostimulatory activity was checked both at the cellular and humoral levels. Cellular immunity was evaluated by neutrophil adhesion test.[24] method was employed for neutrophil adhesion test. Incubation of blood with nylon fibres (NF) produced a decrease in the neutrophil counts due to adhesion of neutrophils to the fibres. Rats of the control group were given 10 ml/kg normal saline, whereas treatment groups were pre-treated with different concentrations of chitosan nanoparticle(150; 300 and 600 mg/kg), peroral for 14 days respectively. On day 14 of chitosan nanoparticle treatment, blood samples were collected by puncturing retro-orbital plexus into heparinized vials and analyzed for total leukocyte cell (TLC) and differential leukocyte cell (DLC) counts. After initial counts, blood samples were incubated with nylon fibers for 15 min at 37° C. The incubated blood samples were again analyzed by TLC and DLC, respectively to give the neutrophil index of blood samples. The percent neutrophil adhesion was calculated by the following formula:

$$\text{Neutrophil adhesion (\%)} = \frac{\text{NIu} - \text{Nit}}{\text{NIu}} \times 100$$

where NIu is the neutrophil index of untreated blood samples and NIt is the neutrophil index of treated blood samples.

Haemagglutinating Antibody (HA) titre

Joshua *et al.*,[24] described the procedure for haemagglutinating antibody titre. The rats of all group were immunized by injecting 0.1 ml of an SRBCs suspension containing 0.5×10^9 cells intraperitoneally on day 0. The rats were divided into control group were given 10 ml/kg normal saline, whereas treatment groups were pre-treated with different concentrations of chitosan nanoparticle (150; 300 and 600 mg/kg),peroral for 7 days respectively. Blood samples were collected from the heart of each rat for serum preparation on the 7th day. The blood samples were centrifuged and serum was obtained. Antibody levels were determined by the hemagglutination technique. Equal volumes of individual serum samples of each group were pooled. Twofold serial dilutions of pooled serum samples made in 25 µl volume of normal saline in microtitration plates were added to 25 µl of 1% suspension of SRBCs in saline. After mixing, the plates were incubated at 37° C for 1 h and thevalue of antibody titre was assigned to the highest serum dilution showing visible haemagglutination.

Delayed-type hypersensitivity (DTH) response

The delayed type hypersensitivity (DTH) response was determined using the method of Allen (2013).[25] The rats of all group were immunized by injecting 0.1 ml of a SRBCs suspension containing 0.5×10^9 cells intraperitoneally on day 0. The rats were divided into control group were given 10 ml/kg normal saline, whereas treatment groups were pre-treated with different concentrations of chitosan nanoparticle(150; 300 and 600 mg/kg), peroral for 8 days respectively. On the 7th day of immunization, all the rats were challenged with SRBC 0.5 x 10^9 cells in the left hind foot pad. The right foot pad was injected with the same volume of normal saline, which served as the control for nonspecific swelling. Increase in footpad thickness was measured 24 h after the challenge.[8]

Phagocytic response (Carbon clearance method)

The method was described by Shingh *et al.*,[26] The rats were divided into control group were given 10 ml/kg normal saline, whereas treatment groups were pre-treated with different concentrations of chitosan nanoparticle(150; 300 and 600 mg/kg), peroral for 7 days respectively. On the 7th day, immediate after the last dose administered to all the rats of each group control as well as treated received an intravenous injection of carbon suspension (1:50 dilution of Indian ink, Hi-Media Laboratories Pvt. Ltd., Mumbai, India) in a dose of 1 ml/200 g body weight. Blood was withdrawn from the retro-orbital venous plexus before injection (0 min) and 15 min after injection of the carbon suspension and 50 µl of blood was lysed with 4 ml of 0.1% sodium carbonate solution (Na2CO3). The optical density was measured spectrophotometrically at 650 nm wavelength.

The results were expressed as a phagocytic index:

K= (In OD *12 min*) – (In OD*0min*) / (t 15*min* – t*0min*)

Where OD12 min and OD0 are the optical densities at time t15 and t0 respectively

Cyclophosphamide-induced myelosuppression

The method described by Bin-Hafeez *et al.*,[27] was employed for cyclophosphamide-induced myelosuppression. Albino rats were divided into five groups designated as a negative control; positive control and treatment groups, each group containing six rats. The negative control group received a saline solution. The positive control group was administered with only cyclophosphamide at the dose of 30 mg/kg, i.p. while treatment groups, rats received cyclophosphamide and varied concentrations of chitosan nanoparticle (150; 300 and 600mg/kg, peroral). The chitosan nanoparticle was given daily for 10 days and cyclophosphamide was injected with cyclophosphamide on the 8th, 9th and 10th day, 1 hr after the administration of the respective treatment. Blood samples were collected on the 11th day of the experiment and analyzed for hematological parameters.

Statistical analysis

Data analysis were performed using SPSS software (SPSS 12.0 K for Windows). All data were expressed as means ± SD values. The ANOVA test was used to test for differences between the groups. Duncan's multiple range test was used to analyze differences between the mean values and differences were considered statistically significant at $P<0.05$.

RESULTS

Effect of chitosan nanoparticle on neutrophil adhesion

The neutrophil adhesion test is an indicative of the marginalization of phagocytic cells in the blood vessels, i.e. an indication of immunostimulation. Incubation of neutrophils with nylon fibers produced a decrease in the neutrophil counts due to adhesion of neutrophils to the fibers. Effect of chitosan nanoparticle on neutrophil activation by the neutrophil adhesion test is shown in (Table 1). The neutrophil adhesion in control group rats was 6.26± 1.85. The chitosan nanoparticle showed a significanti ncrease in neutrophil adhesion at a dose of 300 mg/kg BW and 600 mg/kg BW, but not at doses of 150 mg/kg, when the data were compared with control group rats, suggesting possible immunostimulant action of the chitosan nanoparticle (Table 1).

Table 1: Effect of administration of chitosan nanoparticle on neutrophil adhesion

Group	Neutrophil Index		Neutrophil Adhesion(%)
	UB	FTB	
Control	325.8 ± 31.6	305.4 ± 24.6	6.26 ± 1.85
Chitosan nanoparticle 150 mg/kg	347.6 ± 28.2	326.3 ± 33.7	6.13 ± 2.09
Chitosan nanoparticle 300 mg/kg	384.8 ± 38.8	341.5 ± 29.8	11.25 ± 3.15*
Chitosan nanoparticle 600 mg/kg	429.5 ± 41.4	364.9 ± 33.6	15.04 ± 3.47*

Values are mean ±SD., n=6. * $P < 0.05$ significant; UB: untreated blood; FTB: fiber treated blood.

Effect of chitosan nanoparticle on humoral immunity parameters

The haemagglutination antibody titre was used to assess humoral immune response. The humoral antibody titer value of control group was found to be 28.26± 2.95. Administration of chitosan nanoparticle doses 300 mg/kg BW and 600 mg/kg BW but not at doses of 150 mg/kg produced a significant increase in haemagglutination antibody titre, when the data were compared with control group rats, as an evidence from haemagglutination after incubation of serum with SRBCs (Table 2).

Table 2: Effect of administration of chitosan nanoparticle on haemagglutination titre

Group	HaemagglutinationTitre
Control	28.26± 2.95
Chitosan nanoparticle 150 mg/kg BW	33.51 ± 4.16
Chitosan nanoparticle 300mg/kg BW	41.73 ± 4.69*
Chitosan nanoparticle 600 mg/kgBW	62.15 ± 7.31*

Values are mean±SD., n=6. * $P < 0.05$ significant

Effect of chitosan nanoparticle on Delayed-Type Hypersensitivity (DTH) response

The cell-mediated immune response of chitosan nanoparticle was assessed by Delayed-Type Hypersensitivity(DTH) response, i.e. foot pad reaction. As shown in Table 3, the chitosan nanoparticle produced a significant, *dose-dependent manner* inhibit DTH response in rats. The chitosan nanoparticle at doses 300 mg/kg BW and 600 mg/kg BW but not at dose 150mg/kg BW significantly inhibit DTH reactivity as compared to the control. Inhibition DTH reaction in rats in responserevealed the stimulatory effect of nanoparticle chitosan nanoparticle on T cells (Table 3).

Effect of chitosan nanoparticle on the phagocytic response

The faster removal of carbon particles has been correlated with the enhanced phagocytic activity. The phagocytic activity of the reticular-endothelial system was measured by the removal of carbon particles from the blood circulation. The doses-dependent manner of chitosan

Table 3: Effect of administration of chitosan nanoparticle on Delayed-Type Hypersensi- tivity (DTH)

Group	Delayed-TypeHypersensitivity 24 hr (mm)	Inhibition of DTH (%)
Control	0.73 ± 0.08	-
Chitosan nanoparticle 150 mg/kg BW	0.67 ± 0.05	8.22
Chitosan nanoparticle 300 mg/kgBW	0.46 ± 0.03*	36.98*
Chitosan nanoparticle 600 mg/kg BW	0.31 ± 0.04*	53.85*

Values are mean ±S.D., n=6. * $P < 0.05$ significant

nanoparticle showed a significant increase in the phagocytic index when compared to control indicating that there was the increase in the clearance of colloidal carbon from the blood after administration of these drugs.

The phagocytic index of the control group was 3.88 ± 0.43 (Table 4). Oral administration of chitosan nanoparticle a dose-related increase in the clearance rate of carbon by the cells of the RES. The chitosan nanoparticle showed a significant increase in the phagocytic index at doses of 300 kg/kg BW and 600 mg/kg BW but not at doses of 150 mg/kg, when the data were compared with control group rats, suggesting the phagocytic activity of the chitosan nanoparticle.

Table 4: Effect of administration of chitosan nanoparticle on phagocytic index

Group	Phagocytic Index
Control	3.88 ± 0.43
Chitosan nanoparticle 150 mg/kg BW	4.16 ± 0.81
Chitosan nanoparticle 300 mg/kgBW	5.73 ± 0.79
Chitosan nanoparticle 600 mg/kgBW	7.99± 0.87*

Values are mean ±S.D., n=6. * $P < 0.05$ significant

Effect of chitosan nanoparticle on cyclophosphamide-induced myelosuppression

Cyclophosphamide at the dose of 30 mg/kg, i.p. caused a significant reduction in the hemoglobin, RBCs, WBCs, and platelets count. Combined treatment of cyclophosphamide and nanoparticle extract of chitosan nanoparticlea *dose-dependent manner* result in a restoration of bone marrow activity as compared with cyclophosphamide treatment alone. Significant reduction in white blood cell count was observed in rats treated with cyclophosphamide alone (positive control) as compared to the negative control. The chitosan nanoparticle at doses 600 mg/kg BB but not at doses 150 and 300 mg/kg BW, significantlyincreased the levels of hemoglobin, RBCs, WBCs and platelets count as compared to the positive control treated with cyclophosphamide (Table 5).

DISCUSSION

Recently, it has been reported that the many natural products has immunomodulatory properties and generally act by stimulating both nonspecific and specific immunity. Much natural products used in traditional medicine have immunomodulating activities. Some of these stimulate both humoral and cell-mediated immunity, while others activate only the cellular components of the immune system. The immune system is the vital defense against noninfectious and infectious diseases. A strong mmune system comprises elements that are in balance with one another; if this balance is disturbed, our immune system will be incapable to protect the body against harmful substances.[2,3] Immunomodulation using natural product can provide a substitute to conventional immunotherapy for a range of diseases, especially when host defense mechanism has to be activated under the conditions of impaired immune response. There are several diseases where immunostimulant drugs are needed to overcome the immunosuppression induced by drugs or environmental factors. There is a strong necessity of the drugs that can enhance the immune system to combat the immunosuppressive consequences caused by stress, chronic diseases, and conditions of impaired immune responsiveness. Recently, the natural producthas been commonly used as immunostimulatory.[6] Though natural product have been investigated for diverse pharmacological activities, the immunostimulatory poten-

Table 5: Effect of chitosan nanoparticle on Cyclophosphamide-Induced Myelosuppression

The haematological parameter	Group				
	Control	Cyclophosphamide 30 mg/kg	Chitosan nanoparticle		
			150 mg/kg	300 mg/kg	600 mg/kg
RBC (x10^6/µl)	7.2 ± 0.8	4.2 ± 0.9	4.9 ± 0.8	5.8 ± 1.1	6.9 ± 0.8*
Hb (g/dl)	13.6 ± 1.4	7.6 ± 1.3	8.6 ± 2.1	8.4 ± 1.4	12.3 ± 1.8*
PCV (%)	56.1 ± 5.3	39.3 ± 4.6	42.2 ± 6.5	44.3 ± 7.2	54.2 ± 4.6*
WBC (x10^3/ µl)	6.5 ± 1.1	4.3 ± 0.9	5.1 ± 0.9	5.5 ± 1.3	6.3 ± 0.7*
MCV (fl)	61.3 ± 6.3	46.8 ± 4.2	51.5 ± 6.4	55.5 ± 9.1	59.7 ± 5.6*
MCHC (g/dl)	36.5 ± 4.9	20.6 ± 5,1	24.6 ± 3.9	29.8 ± 5.1	34.1 ± 4.8*
Lymphocyte (%)	64.8 ± 7.3	43.9 ± 5.8	51.5 ± 7.8	56.6 ± 8.9	62.2 ± 7.1*
Monocyte (%)	3.6 ± 0.6	1.8 ± 0.6	2.1 ± 0.6	2.9 ± 0.8	3.4 ± 0.6*
Neutrophils (%)	1.6 ± 0.4	0.8 ± 0.3	0.7 ± 0.5	0.9 ± 0.4	1.2 ± 0.5*

*Significant difference between the chitosan nanoparticlegroups and the control group ($P < 0.05$).Values are expressed as mean ±SD.The data represent the average from 6 rats

tial of chitosan nanoparticle still remains unknown. In this result of the study, we showed that chitosan nanoparticle had immunomodulatory activity in experimental models of cellular and humoral immunity. The study was carried out using four different methods, each of which provides information about the effect on different components of the immune system. The results of the present study indicate that chitosan nanoparticle is a potent immunostimulant, stimulating both specific and nonspecific immune mechanisms.

The neutrophil adhesion to nylon fibres describes the margination of polymorpho nuclear lymphocyte in the blood vessels and the number of macrophages reaching the site of inflammation. Neutrophils are an important component of the innate immune system, with the main role in the clearance of extracellular pathogens. Both localization and neutralization of microorganisms are functions of neutrophil that are regulated by specific inflammatory mediators released from the site of infection. The neutrophil, an end cell unable to divide and capable ofa wide range of responses, in particular, chemotaxis, phagocytosis, exocytosis and both intracellular and extracellular killing.[18] In the present study, nanoparticle extract ofchitosan nanoparticle evoked asignificant increase in percent neutrophils. This may potentially help in increasing immunity of body against microbial infections.

The indirect haemagglutination test was performed to confirm the effect of chitosan nanoparticle on the humoral immune system. It is composed of interacting B cell with antigens and subsequently proliferating and differentiating into antibody-producing cells. The chitosan nanoparticle can stimulate the humoral immune response to SRBCs, which was evidenced by an increase in the antibody titre in rats indicated the enhanced responsiveness of T and B lymphocyte subsets, involved in the antibody synthesis. The high values of haemagglutinating antibody titre of chitosan nanoparticle shown that immunostimulation was achieved through humoral immunity. B lymphocytes and plasma cells function in the humoral immunity component of the adaptive immune system by secreting antibodies such as IgG and IgM are the major immunoglobulins which are involved in the complement activation, opsonization, neutralization of foreign bodies.[28]

Cell-mediated immunity (CMI) involves effectors mechanisms carried out by T lymphocytes and their products (lymphokines). CMI responses are critical to defense against infectious microorganisms, infection of foreign grafts, tumor immunity, and delayed-type hypersensitivity reactions.[2,4] Therefore, increase in DTH reaction in rats in response to T cell-dependent antigen revealed the stimulatory effect of chitosan nanoparticle on T cells. In the present study, chitosan nanoparticle showed an overall stimulatory effect on the immune functions in rats. Stimulatory effects were observed on both humoral and cellular immunity. In DHT test, the chitosan nanoparticle showed an increase response in all doses, but this increase was significant only in dose 600 mg/kg. This activity could be due to the chitosan augment the humoral response, by stimulating the macrophages and B lymphocytes subsets involved in antibody synthesis. The mechanism behind this elevated DTH during the CMI responses could be due to sensitized T-lymphocytes. When challenged by the antigen, they are converted to lymphoblast and secrete a variety of molecules including proinflammatory lymphokines, affecting more scavengers cells to the site of reaction.[4] An increase in DTH response indicates a stimulatory effect of the chitosan nanoparticle which has occurred on the lymphocytes and accessory cell types required for the expression of this reaction.[5]

The carbon clearance test was used to investigate the activity of drugs on the reticulo endothelial system. The reticuloendothelial system (RES) is a diffuse system consisting of phagocytic cells. Cells of the RES play a vital role in the clearance of particles from the bloodstream.[26] When colloidal carbon particles in the form of ink are injected directly into the systemic circulation, the rate of clearance of carbon from the blood by macrophage is governed by an exponential equation. Phagocytosis is the process by which certain body cells, collectively known as phagocytes, ingests and removes microorganisms, malignant cells, inorganic particles and tissue debris.[8,24] Chitosan nanoparticle showed remarkable augmentation in the phagocytic indexa dose-related increase in the clearance rate of carbon by the cells of the reticulo endothelium system, it is speculated that it might be due to increasing in the activity of the reticulo endothelial system by prior treatment of animals with Chitosan nanoparticle

The administration of chitosan nanoparticle significantly the total WBC count, RBCs count, hemoglobin and platelets count and also restored the myelosuppressive effects induced by cyclophosphamide. The results of the present study indicate that the chitosan nanoparticle can stimulate the bone marrow activity. The bone marrow is the organ most affected during any immunosuppression therapy and a sensitive target particularly to cytotoxic drugs such as cyclophosphamide. Loss of stem cells and the inability of the bone marrow to regenerate new blood cells results in thrombocytopenia and leucopenia.[27] Administration of the chitosan nanoparticle was found to increase the total WBC count, which was lowered by cyclophosphamide, a cytotoxic drug, indicating that the chitosan nanoparticle can stimulate the bone marrow activity.

CONCLUSION

It could be concluded that dose-dependent manner chitosan nanoparticle may stimulate both cellular and humoral immune responses. The chitosan nanoparticle not only potentiates nonspecific immune response but also improve humoral as well as cell-mediated immunity effectively. The effectiveness of chitosan nanoparticle treated animals in overcoming the side effects of drug-induced myelosuppression provides sufficient evidence for balancing and adaptogenic efficacy of the chitosan nanoparticle. Thus, from the results obtained, it can be concluded that chitosan nanoparticle has therapeutic potential and could be served as an effective immunostimulatory candidate. Further studies on the mechanism of action of chitosan nanoparticle, in order to establish its therapeutic potential for the prevention of autoimmune diseases, are planned in the laboratory.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

ABBREVIATIONS

SRBC: Sheep Red Blood Cells; **DTH:** Delayed-Type Hypersensitivity; **HA:** Haemagglutinating Antibody; **RBC:** Red Blood Cell; **WBC:** White Blood Cell; **PCV:** Packed Cell Volume; **MCV:** Mean corpuscular volume; **MCHC:** Mean Corpuscular Hemoglobin Concentration; **CMI:** Cell-Mediated Immunity

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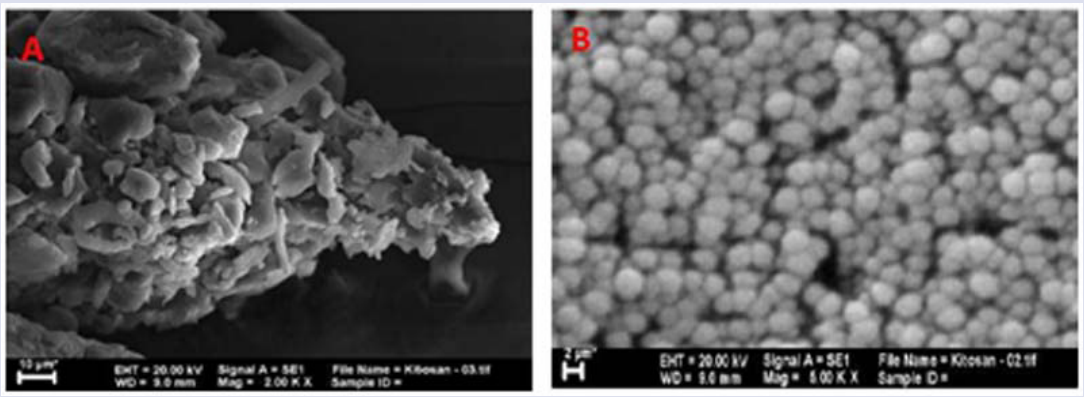
GRAPHICAL ABSTRACT



SUMMARY

- The chitosan nanoparticle a doses-dependent manner induced a significant increase in percent of neutrophil adhesion fibers as well as the increase in haemagglutinating antibody titre values and potentiated the delayedtype hypersensitivity reaction induced by sheep red blood cells. Also, it prevented myelosuppression in cyclophosphamide drug-treated rats and good response towards phagocytosis in carbon clearance assay. It could be concluded that chitosan nanoparticle has immunotherapeutic potential and could be served as an effective immunostimulatory candidate.

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