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Adjuvant effect of biopolymeric fraction from Picrorhiza kurroa to promote both Th1 and Th2 immune responses

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Abstract

In this study, the plant based biopolymeric fraction RLJ-NE-205 isolated from Picrorhiza kurroa were evaluated for its potential ability as an adjuvant effect on the immune responses to Ovalbumin (specific antigen) in mice. Immunization in Balb/C mice with variable doses of RLJ-NE-205 (10 – 80 μg) with optimized dose of Ovalbumin (100 μg) dissolved in phosphate buffered saline or containing alum (200 μg) on Days 1 and 15. After two weeks of the challenging dose, OVA specific antibodies in serum, spleen cell proliferation assay, estimation of Th1 (IL-2/IFN-gamma) and Th2 (IL-4) cytokines in serum, CD3/CD4/CD8/CD19 surface markers of T and B cell, costimulatory molecules (CD80/CD86) in spleen cells and safety studies were observed. The results suggest that OVA mediated specific antibody levels (IgG, IgG1 and IgG2a) in serum were significantly enhanced by RLJ-NE-205 (20 μg) containing 100 μg OVA compared with OVA control group. Moreover, RLJ-NE-205 (20 μg) containing 100 μg OVA on IgG, IgG1, and IgG2a antibody responses to OVA in mice were more significant than those of alum. RLJ-NE-205 (20 μg) significantly enhanced the OVA induced spleen cell proliferation and estimation of Th1 and Th2 cytokines in the OVA immunized mice. However, no significant differences were observed among the OVA group and OVA/alum group. At the dose of 100 μg OVA containing 20 μg RLJ-NE-205, there was a significant increase in the Th1 and Th2 cytokines in serum and CD3/CD4/CD8/CD19 surface markers and costimulatory molecules (CD80/CD86) in the spleen cell by flow cytometry were observed.

Keywords: Picrorhiza kurroa; Ovalbumin; Alum; RLJ-NE-205.

Introduction

A body of evidence showed that numerous bioactive polysaccharides isolated from herbal plants have immunomodulation and anti-cancer effects. Previous studies have shown that polysaccharides evoke stronger immune responses than alum or others [1, 2, 3, 4, 5]. Thus, the polysaccharides from medicinal herbs are becoming an attractive material as pharmaceutical products and may provide an opportunity to develop a new adjuvant for vaccine antigen. Indeed, a wide range of bioactive polysaccharides have been isolated from various medicinal plants and these polysaccharides have been shown to possess immunomodulatory activity through their ability to modulate macrophage function [3, 4]. Appropriate enhancement of innate immune functions by bioactive compounds can then augment host defense responsiveness because of their minute toxicity and high potency of these molecules, plant polysaccharides represent one of the ideal or best candidates for therapeutics with immunomodulatory action.

Picrorhiza kurroa is a well known herb in the Ayurvedic system of medicine (belongs to the family Scrophulariaceae) and has traditionally been used to treat disorders i.e. liver, upper respiratory tract, scorpion sting etc [6, 7]. The leaves of the Picrorhiza kurroa are flat, oval and sharply serrated and flowers generally appeared from June to August and the manual harvesting of the plant from October to December. Most of the active constituents are generally obtained from the root and rhizomes. In our previous paper, we isolated active polysaccharide fraction (RLJ-NE-205) from Picrorhiza Kurroa that induce both humoral and cellular arms of the immune system in Balb/C mice [8]**.** Similarly, a number of polysaccharides that provoke immune responses and anti-tumor activities via macrophage activation have been purified from higher plants and mushrooms [9], [10], [11] and [12]. A better understanding of the biology of T cell

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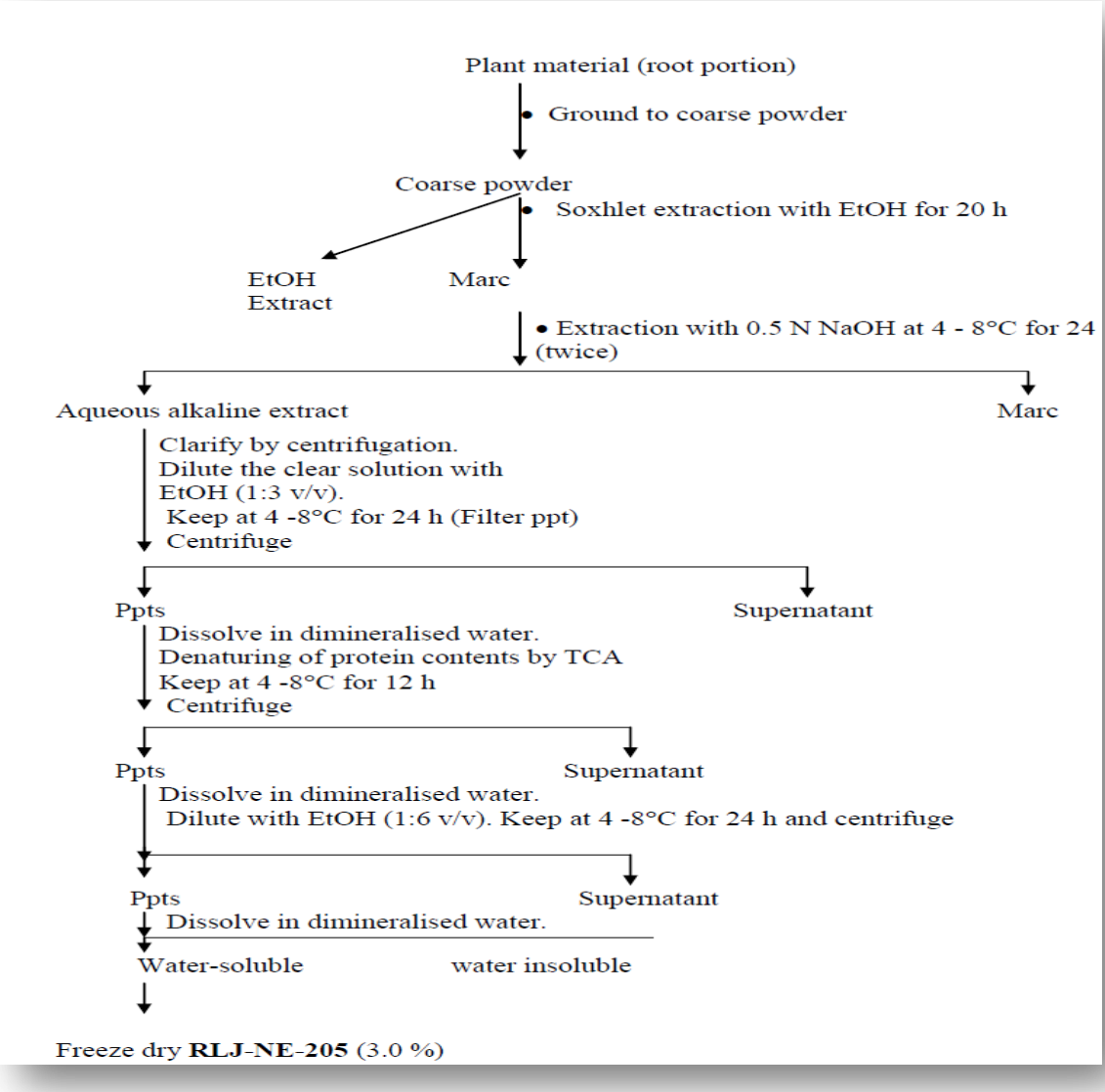
subpopulations viz. CD4/CD8, T and B cell memory, regulatory T cells and mucosal immunity has profound implications for a modern approach to adjuvant screening and development. The present study was undertaken to validate the immunoadjuvant effects of RLJ-NE-205 against weak antigen ovalbumin.

Materials and Methods

Preparation of Biopolymeric fraction RLJ-NE-205 from Picrorhiza kurroa

The organic solvent exhausted material (0.5 kg) of the plant Picrorhiza kurroa was soaked in 2 M aqueous sodium hydroxide and kept at 4 °C overnight. The extract was filtered and the alkaline solution centrifuged at 6000 – 7000 r. p.m. at 4 °C. The above process was repeated and the aqueous alkaline solution was pooled with the first extract. The combined extracts were diluted with alcohol (1: 6) and kept overnight (4 °C). The precipitate was collected through centrifugation at 6000 – 7000 r. p.m. and dissolved in distilled water (400 ml), acidified with equal volume of 15 % aq. TCA and kept overnight at 4 °C. The precipitated biopolymeric fraction coded as RLJ-NE-205 (3.5 g) obtained by centrifugation was suspended in warm distilled water (500 ml) and centrifuged. The aqueous solution was lyophilized and fraction RLJ-NE-205 (3.0 g) was obtained as an amorphous solid. From HPLC of the hydrolysed (2N - TFA) biopolymeric fraction RLJ-NE-205 in comparison to authentic monosaccharides it was observed that biopolymeric fraction is composed of glucose, xylose, galactose and arabinose in the molar ratio of 3.0, 1.0, 1.8 and 4.1. A flow sheet is represented as shown in **Fig.1**.



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Animals

Male adult Balb/C mice 10 – 12 weeks old and weighing 20 – 22 g obtained from the animal house of the Indian Institute of Integrative Medicine (IIIM), Jammu in groups of 5 - 10 were employed for the study and kept in plastic cages. These were maintained at a room temperature of 22 ± 2 °C with 12 h light/dark cycle and free access to pellet food (Lipton India limited) and water. The ethical committee of the Indian Institute of Integrative Medicine (IIIM) instituted for animal handling approved all protocols.

Immunization

In order to find out the adjuvant activity of biopolymeric fraction RLJ-NE-205 with ovalbumin antigen. Animals were immunized s.c. with 100 µg OVA formulated with one of the following delivery vehicles: Phosphate buffered saline and RLJ-NE-205 (10, 20, 40 and 80 µg) on Day 1. Saline-treated animals were included as controls. A challenging injection was given two weeks later. Control adjuvant (standard) was alum (200 µg). Alum was formulated with OVA at a ratio of two parts adjuvant to one part antigen immediately prior to administration. The resultant formulation was thoroughly vortexed before use. In every experiment, one group was kept as control, whilst another group received standard drug for comparison of the study and authenticity of the experiment.

Assay of IgG (anti-OVA) titre in the sera of mice by Elisa

OVA specific IgG antibodies in serum were detected by an indirect ELISA [13]. In brief, microtiter plate wells were coated with 100 µl OVA solution (50 µg/ml) for IgG antibodies in 50 mM carbonate-bicarbonate buffer, pH 9.6) for 24 h at 4 °C. The wells were washed three times with PBS containing 0.05 % (v/v) Tween 20 (PBS/Tween) and then blocked with 5 % FCS/PBS at 37 °C for 1 h. After three washings, 100 µl of diluted serum sample (IgG, 1: 400) or 0.5 % FCS/PBS as control was added to duplicate/triplicate wells. The flat bottom 96 well Elisa plates were then again incubated for additional 1 h at 37 °C, followed by three times of washing. Add rabbit anti-mouse IgG horse radish peroxidase conjugate (100 µl) diluted 1 : 1000 with 0.5 % FCS/PBS were added. Elisa plates were further incubated for 1 h at 37 °C. After washing, the peroxidase activity was assayed as following: 100 µl of substrate solution (10 mg of o-phenylenediamine and 37.5 µl of 30 % H_2O_2 in 25 ml of 0.1 M citrate-phosphate buffer, pH 5.0) was added. The flat bottom Elisa plate plate was again incubated for additional 10 min at 37 °C and then suddenly stop solution was added. The optical density (OD) was measured in an ELISA reader at 450 nm.

Assay of IgG1 and IgG2a (anti-OVA) titre in the sera of mice by Elisa

OVA specific IgG1 and IgG2a antibodies in serum were detected by an indirect ELISA [13]. In brief, microtiter plate wells were coated with 100 µl OVA solution (25 and 50 µg/ml) for IgG1 and IgG2a antibodies, respectively, in 50 mM carbonate-bicarbonate buffer, pH 9.6) for 24 h at 4 °C. The wells were washed three times with PBS containing 0.05 % (v/v) Tween 20 (PBS/Tween), and then blocked with 5 % FCS/PBS at 37 °C for 1 h. After three washings, 100 µl of diluted serum sample (IgG1, 1:100; IgG2a, 1:200) or 0.5 % FCS/PBS as control was added to duplicate/triplicate wells. The flat bottom 96 well Elisa plates were then again incubated for 1 h at 37 °C, followed by three times of washing. Aliquots of 100 µl of goat anti-mouse IgG1 peroxidase conjugate 1: 500 or IgG2a peroxidase conjugate 1: 100 with 0.5 % FCS/PBS were added. The flat bottom 96 well Elisa plates were further incubated for 1 h at 37 °C. After washing, the peroxidase activity was assayed as following: 100 µl of substrate solution was added to each well. The plate was incubated for 10 min at 37 °C, and enzyme reaction was terminated by adding 50 µl/well of 2 N H_2SO_4. The OD was measured in an ELISA reader at 450 nm.

Splenocyte proliferation assay (ex vivo)

Mice were sacrificed by carbon dioxide anesthesia. The spleens were excised aseptically and lymphocytes isolated. Briefly, single cell suspensions were prepared by teasing the tissue between two glass slides and cells were centrifuged at 1800 rpm for 10 min at 4 °C. Erythrocytes present were lysed with red cell lysis buffer for 5 min. Lymphocytes obtained were then washed thrice with PBS. Count the number of cells using haemocytometer and determined by trypan blue dye exclusion technique. Cell viability exceeded 95 % [14].

To evaluate the effect of biopolymeric fraction RLJ-NE-205 on the proliferation of splenic lymphocytes [8], spleen cell suspension (2×10^6 cell/ml) was pipetted into 96 well plates (200 µl/well) in the presence of OVA (5 µg/ml) cultured at 37 °C for 48 h, the plates were centrifuged at 1800 rpm, 5 min and the supernatant was collected for the estimation of cytokines in cell culture supernatant and add fresh 100 µl fresh complete media in 96 well plate and again incubate for 24 h and then add 20 µl of MTT solution (5 mg/ml) were added to each well and incubated for 4 h. The plates were centrifuged (1400 × g, 5 min) and the untransformed MTT was removed carefully by pipetting. Add 100 µl of a DMSO was added and the absorbance was evaluated in an ELISA reader at 570 nm after 15 min.

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Estimation of Th1 and Th2 cytokines in serum

Cytokine concentrations in the serum were determined by ELISA kits that were specific against murine cytokines. Levels of Th1 (IFN-γ, IL-2) and Th2 (IL-4) cytokines were measured using ELISA (Quantikine, ELISA kits). Assays were performed according to the manufacturer's instructions [15, 16].

Lymphocyte immunophenotyping (CD3/CD4/CD8/CD19) and costimulatory molecules estimation in spleen

The percentages of T lymphocyte subsets in the spleen were measured using flow cytometric analysis [8]. Spleen single cell suspensions were dually labeled with FITC-conjugated and PE- conjugated antibodies.

The spleen (1/3 of the organ) was placed in PBS buffer (without Mg^{2+} and Ca^{2+}) stored on ice prior to preparation of single cell suspensions. Splenic erythrocytes (RBC's) were lysed with FACS lysing solution/red blood cell lysing buffer/ACK lysis buffer. Cell suspensions were refrigerated at 4°C pending staining with antibodies. For each sample, 2 x 10^6 cells were stained with conjugated anti-CD80 (B7-1), anti-CD3 & CD4 FITC and anti-CD86 (B7-2), anti-CD8a & CD19 PE antibodies. After staining with flow cytometric antibodies, cells were washed two to three times with wash buffer and finally resuspended in PBS for flow cytometric analysis. The forward and side scatter gating applied for data acquisition of cell surface markers on 10,000 events and fraction of cell populations representing different phenotypes analyzed using cell quest software (FACS calibur, Becton Dickinson).

Safety studies

Safety of the active adjuvant candidate with OVA was proven by injecting it intraperitoneally into mice. Balb/c mice were divided into groups of 10 animals each and were inoculated with variable doses of RLJ-NE-205 and OVA, which had been resuspended in PBS. Control mice, received PBS. After injection, mice were observed daily for a period of 8 weeks. Deviations from their normal behavior or reactions at the injection site were recorded. The weight of the mice was taken on days 0, 24, 28 and 60 post-injection.

Statistical analysis

Data were expressed as mean ± S.E. and statistical analysis was carried out using one-way ANOVA (Bonferroni correction multiple comparison test).

Results

Effect of biopolymeric fraction RLJ-NE-205 on humoral response against OVA-specific antibody viz. IgG, IgG1 and IgG2a titre

OVA specific IgG, IgG1 and IgG2a antibody levels in the sera were measured two weeks after the last immunization, there is a significant enhancement in total serum IgG, IgG1 and IgG2a levels were observed (**Fig.2**) in the group of biopolymeric fraction RLJ-NE-205. The impact of RLJ-NE-205 on the production of IgG isotype viz. IgG1 and IgG2a was monitored in sera of the animals. OVA immunized animals given different doses of RLJ-NE-205 showed dose dependent increase in the secretion of IgG, IgG1 and IgG2a, with maximum response at 20 μg on day 15^{th} and 28^{th} and there was significantly higher when compared to the value of OVA group. There were significant differences among the total serum IgG2a levels in mice immunized with RLJ-NE-205. Alum (200 μg) the standard adjuvant taken for comparison and authentication of the experimentation, increased IgG and IgG1 titre but poorly elicited IgG2a titre when compared to the value of OVA control group. Thus, findings indicate that biopolymeric fraction RLJ-NE-205 could significantly enhance serum antibody viz. IgG, IgG1 and IgG2a production as compared with alum.

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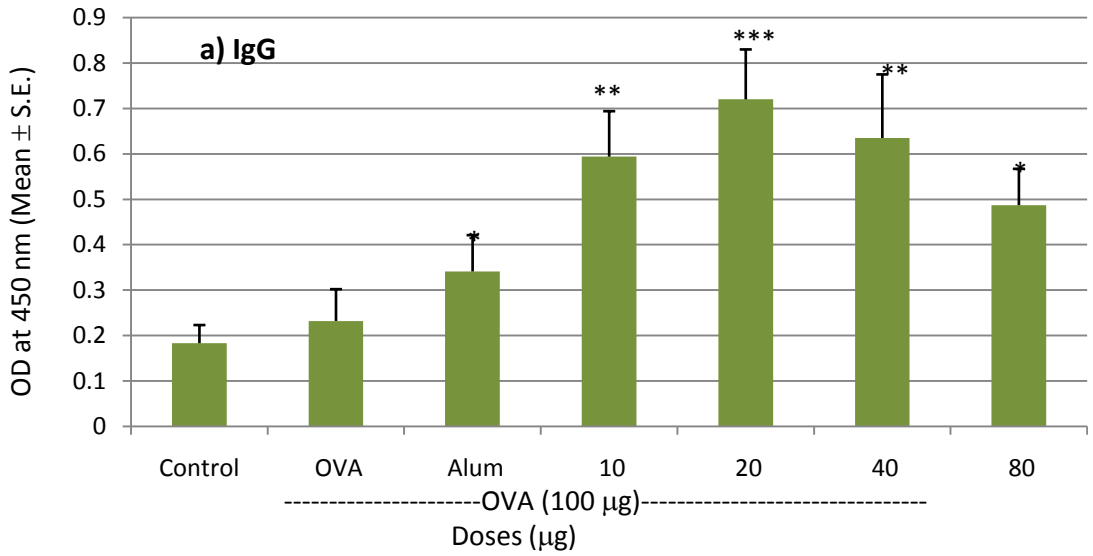

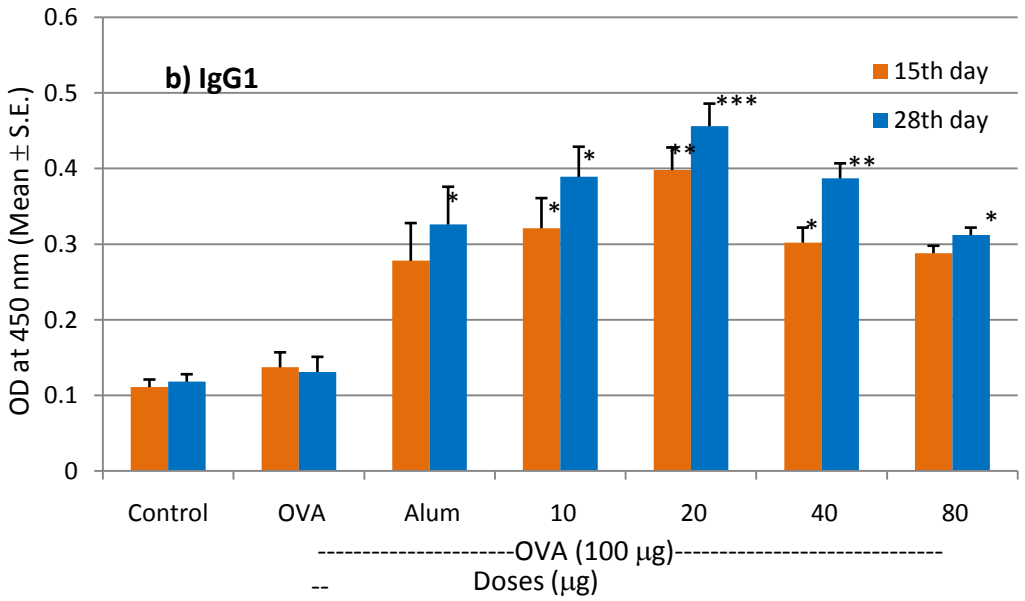

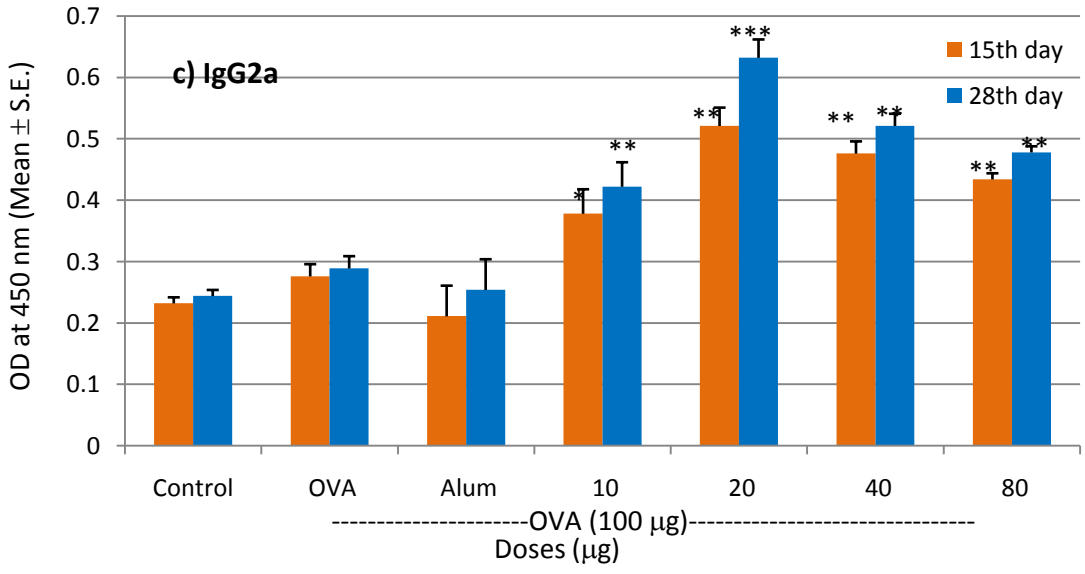

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Fig.2. Effect of biopolymeric fraction RLJ-NE-205 on OVA specific IgG, IgG1 and IgG2a antibody titre

Groups of ten male Balb/c mice were immunized s.c. with OVA 100 µg alone or with OVA 100 µg dissolved in saline containing alum (200 µg) or RLJ-NE-205 (10, 20, 40 and 80 µg) on Days 0 and 15. Sera were collected two weeks after the last immunization. OVA-specific IgG, IgG1 and IgG2a antibodies in the sera were measured by an indirect ELISA method as described in materials and methods. The values are presented as mean ± S.E. and significant differences with control were designated as *P < 0.05, **P < 0.01, ***P < 0.001.

Effect of biopolymeric fraction RLJ-NE-205 on cell mediated immune response against splenocyte proliferation assay (ex vivo)

The effect of biopolymeric fraction RLJ-NE-205 on OVA stimulated splenocyte proliferation in mice immunized with OVA is shown in **(Fig. 3).** The results showed that biopolymeric fraction RLJ-NE-205 significantly enhanced the cell mediated immune response when compared to the OVA control group. RLJ-NE-205 at 20 µg significantly enhanced proliferative response to OVA was observed when compared to the control group. Alum (200 µg) the standard adjuvant used in this test model, enhanced proliferative response to OVA when compared to the control animals. The results indicated that biopolymeric fraction RLJ-NE-205 significantly enhanced the splenocyte proliferation assay as compared with alum.

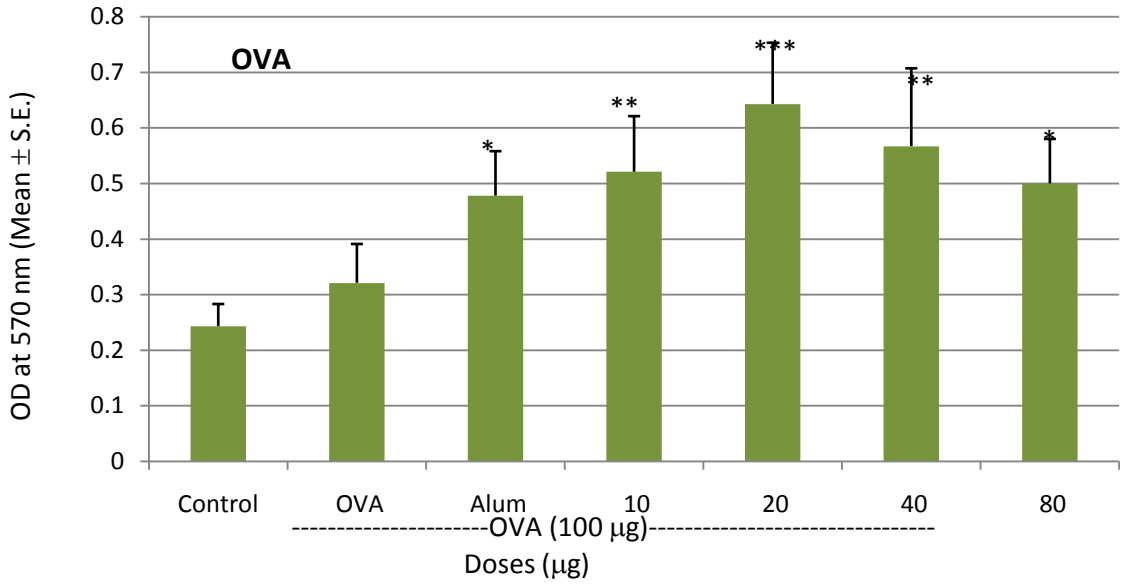

Fig.3. Effect of biopolymeric fraction RLJ-NE-205 on OVA stimulated splenocyte proliferation assay (ex vivo)

Splenocytes were prepared two weeks after the last immunization and cultured with OVA (5 µg/ml) for 72 h. Splenocyte proliferation was measured by the MTT method as described in the materials and methods. Values are presented as mean ± S.E. and significant differences with control were designated as *P < 0.05, **P < 0.01, ***P < 0.001 when compared to the value of control.

Effect of biopolymeric fraction RLJ-NE-205 on estimation of Th1 (IL-2/IFN-γ) and Th2 (IL-4) cytokines in serum

The effect of biopolymeric fraction RLJ-NE-205 on Th1 (IL-2/IFN-γ) and Th2 (IL-4) cytokines in mice immunized with OVA is shown in (**Fig. 4**). The results showed that biopolymeric fraction RLJ-NE-205 significantly enhanced the Th1 (IL-2/IFN-γ) and Th2 (IL-4) cytokines when compared to the OVA control group**.** However, production of IL-2, IL-4 and IFN- γ level in serum was numerically higher in the mice immunized with 20 µg RLJ-NE-205 than those of the mice immunized with OVA control group. However, no significant differences were observed between the OVA control group and OVA/alum. Alum (200 µg) the standard adjuvant, increased Th2 (IL-4) but inhibited the cytokine (IL-2 and IFN-γ) level in serum. The results indicated that biopolymeric fraction RLJ-NE-205 increased the cytokine (IL-2 and IFN-γ) and Th2 (IL-4) level in serum as compared with alum and control group.

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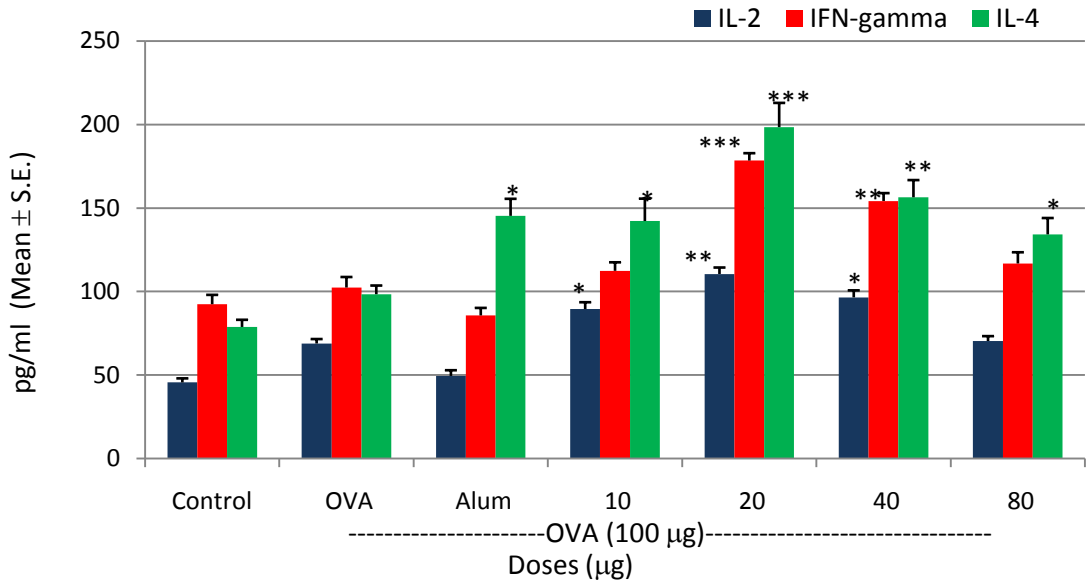

Fig.4. Effect of biopolymeric fraction RLJ-NE-205 on Th1 and Th2 cytokine profile in serum

Groups of ten male Balb/c mice were immunized s.c. with OVA 100 µg alone or with OVA 100 µg dissolved in saline containing alum (200 µg) or RLJ-NE-205 (10, 20, 40 and 80 µg) on Days 0 and 15. Sera were collected two weeks after the last immunization for the estimation of Th1 (IL-2 and IFN-gamma) and Th2 (IL-4) in serum. The results are presented as Mean ± S.E.. P values: *P < 0.05, **P < 0.01, ***P < 0.001 when compared to the value of control.

Effect of biopolymeric fraction RLJ-NE-205 on lymphocyte immunophenotyping (CD3/CD4/CD8/CD19) and co-stimulatory molecules in spleen

The effect of biopolymeric fraction RLJ-NE-205 on $CD3^+$ /$CD4^+$/$CD8^+$ T and $CD19^+$ B cells in the spleen cells from the OVA immunized mice were shown in **(Table 1)**.

TABLE 1:- Effect of biopolymeric fraction RLJ-NE-205 on spleen T cell subtypes (CD4 AND CD8)

S.NO.	Treatment	Doses (µg)	Spleen T-cell subtypes (% Gated) CD4+CD8-	CD4-CD8+
1	Control	-	14.2 ± 1.7	9.6 ± 1.3
2	Alum	200	24.2 ± 1.7**	10.3 ± 0.7
3	RLJ-NE-205	10	20.5 ± 0.8	11.0 ± 0.3
4	RLJ-NE-205	20	28.6 ± 0.9***	15.7 ± 1.0***
5	RLJ-NE-205	40	26.8 ± 1.6**	13.1 ± 0.78**
6	RLJ-NE-205	80	19.2 ± 1.3	10.6 ± 1.1

Flowcytometric analysis of T cell surface markers expression in splenocytes of RLJ-NE-205 treated mice. Cells were incubated with anti-mouse FITC-labeled CD4 and PE conjugated CD8 monoclonal antibodies. Data are Mean ± S.E. (n = 10). P values: *P < 0.05, **P < 0.01, ***P < 0.001 when compared to the value of control.

Although the proportions of CD3/CD4/CD8 T cell estimation in the spleen cells from the mice immunized with 20 µg RLJ-NE-205 and alum were higher than those from the OVA control group. CD3/CD19. Similarly, the proportions of CD19 B cell estimation in the spleen cells from the mice immunized with 20 µg RLJ-NE-205 were higher than those from the OVA control group as shown in **Fig. 5.**

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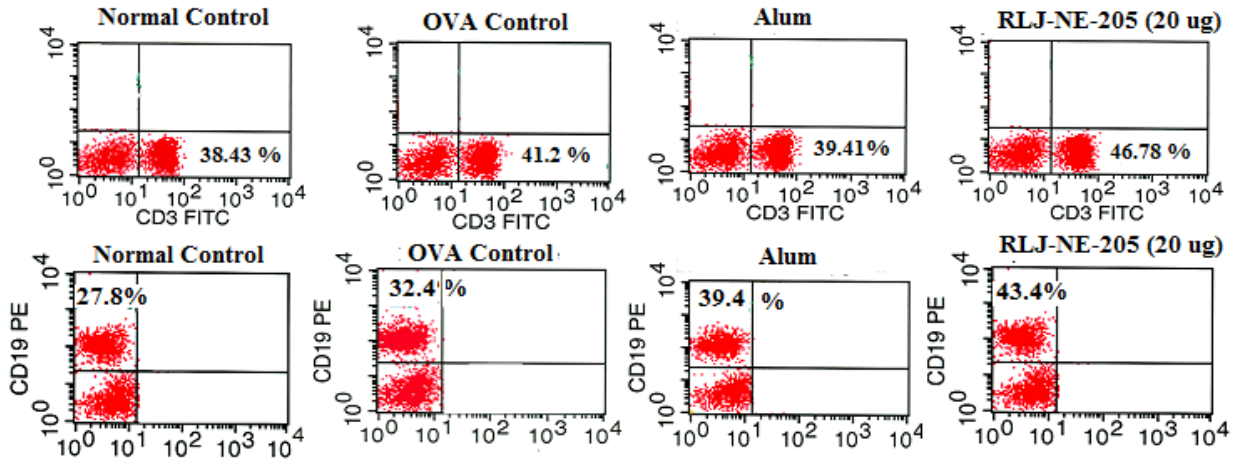

Fig.5. Effect of biopolymeric fraction RLJ-NE-205 on T (CD3) and B (CD19) cell surface markers on spleen cells by flow cytometry

Flowcytometric analysis of T cell surface markers expression in splenocytes of RLJ-NE-205 treated mice. Cells were incubated with anti-mouse FITC-labeled CD3 and PE conjugated CD19 monoclonal antibodies

In addition, Biopolymeric fraction RLJ-NE-205 significantly upregulated the costimulatory expression of both CD80 and CD86 on splenic macrophages in a dose-dependent manner. Biopolymeric fraction RLJ-NE-205 at the dose of 20 µg (optimum dose range) induced optimum enhancement of 2-fold in the number of cells expressing CD80/CD86 over the control animals (**Fig.6**)

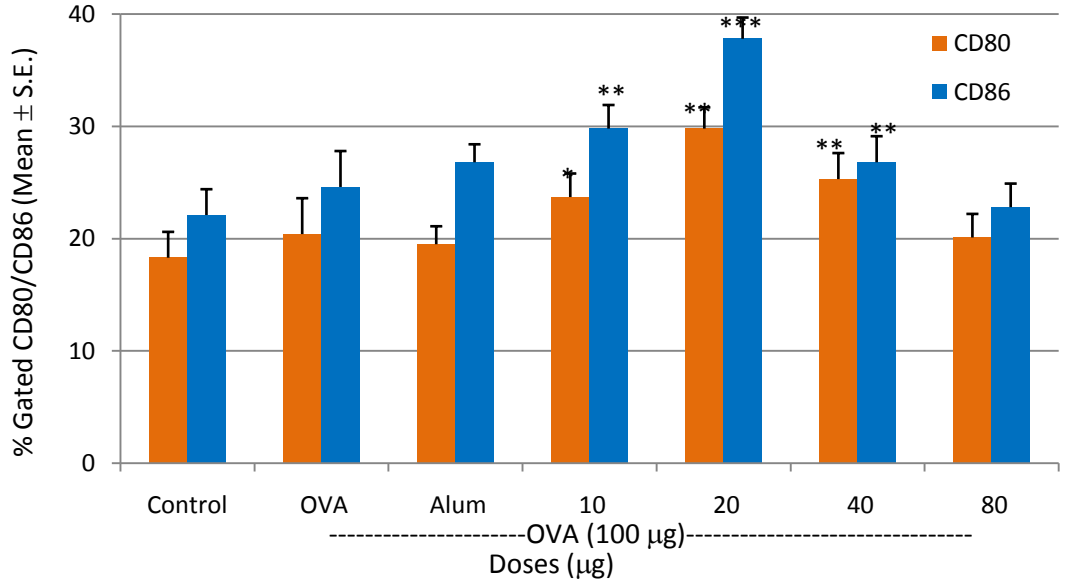

Fig.6. Effect of biopolymeric fraction RLJ-NE-205 on co-stimulatory molecules CD80 (B7-1) and CD86 (B7-2)

Flow cytometric analysis of the expression of co-stimulatory signal molecules in spleen-derived macrophages. Data represented by percent CD80/CD86 positive cell populations are mean ± S.E. (n=5); *P < 0.05, **P < 0.01, ***P < 0.001 (control vs. RLJ-NE-205-treated groups; one-way ANOVA followed by Bonferroni multiple comparison test).

Safety Studies

No weight loss observed for mice when recorded on days 0, 15 and 28 post-injection. Mice were kept under observation for 28 days post-injection for any toxic manifestation and no visible symptoms or any other undesirable effects was observed after the treatment of biopolymeric fraction RLJ-NE-205. In our studies, 8 animals were taken up for safety

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studies. These animals were kept in an isolator at a temperature of 25 ^{o}C. And the rectal temperature was measured twice daily. Animal body weights were taken on 0, 7, 14 and 28th day. Even though the fluctuation in body weights has been observed (data not shown), no significant fluctuation in body temperature was observed.

Discussion

Adjuvant research is a field that is advancing rapidly, reflecting high rate at which new adjuvants are being discovered. The requirement of a safe and effective adjuvant for current vaccines cannot be overstated. Newly developed vaccines are usually based on target antigen i.e specific and non-specific, they are usually weak immunogenic, costly/expensive and induce poor immunopharmacological responses. However, plant based adjuvants can override such immunological inadequancy and help in mounting protective humoral and cellular immune responses. There is a significant interest in developing vaccine adjuvant that combine the safety advantages of subunit or recombinant protein based vaccines with enhanced efficacy. However, many of them are not highly immunogenic whether administered parenterally or mucosally and approved adjuvants are ineffective in triggering immunity, thus identification of plant based adjuvant capable of facilitating antigen delivery to immune responsive cells and functioning as adjuvants.

OVA is commonly used as a model for immunogen specific T and B cell mediated immune function. Biopolymeric fraction RLJ-NE-205 (20 μg) significantly enhanced OVA specific IgG and its isotypes IgG1 and IgG2a titres were observed in group of immunized mice compared with OVA control and standard alum group. It is generally known that Con A stimulates T cell proliferation [17, 18]. When splenocytes separated from mice immunized with OVA were exposed to RLJ-NE-205, it is possible that lymphocytes recognize and receive second signals and test candidate viz. biopolymeric fraction RLJ-NE-205 could facilitate OVA specific lymphocytes to proliferate and differentiate into effector cells and memory cells [19].

Cell mediated immunity, mediated by T lymphocytes, play an important role to combat intracellular infections. Among the T lymphocytes, helper T cells induce B lymphocytes to secrete antibodies and cytotoxic T lymphocytes help phagocytes to destroy infection induced by pathogen and to kill intracellular microbes. The capacity to elicit an effective T cell immunity can be shown by the stimulation of lymphocyte proliferation response and cytokine estimation. The results indicated that RLJ-NE-205 could significantly increase the activation potential of T (IgG2a) and B (IgG1) cells in OVA-immunized mice. RLJ-NE-205 also had a significant stimulatory effect on CD4+ and CD8+ T cells, thereby confirming its general effect on the cell-mediated immune response. Furthermore, co-stimulatory signals are the most important secondary signals playing an important role in the cell–cell cross-talk for the delivery of the required immune response. CD80 has been found to play key role in maintaining the state of immune response, where as another co-stimulatory molecule i.e. CD86 plays a role in maintaining immune memory [20]. The expressions of CD80 and CD86 on splenic macrophages of RLJ-NE-205-treated mice were found significantly enhanced supporting its potential of activating the antigen presenting cells through co-stimulatory signals that eventually help in the generation of effective immune response by secreting various signal molecules like cytokines and chemokines.

Meanwhile, cytokine measurement also revealed that RLJNE-205 significantly promoted the production of the Th1 (IL-2 and IFN-γ) and Th2 (IL-4) cytokines in OVA immunized mice. These results suggested that RLJ-NE-205, be able to simultaneously stimulate Th1 (IgG2a) and Th2 (IgG1) immune response. In our conditions, RLJ-NE-205 enhanced significantly a specific antibody and cellular response against OVA in mice.

In conclusion, two important features of RLJ-NE-205 have been clearly shown: (1) RLJ-NE-205 is a strong Th1/Th2 adjuvant for the OVA in the mice model and (2) the immunogenicity of the RLJ-NE-205-adjuvanted OVA is better than that of OVA adjuvanted with alum. The OVA-specific antibody immune response induced by RLJ-NE-205 was associated with higher antibody titers than that provided by the alum vaccine and moreover cell-mediated responses were elicited. Furthermore, RLJ-NE-205 also significantly enhanced the population of cell surface marker CD4/CD8 and co-stimulatory molecules CD80/CD86. Thus, RLJ-NE-205 is a potent enhancer of antigen-specific humoral and cell-mediated immune responses, thus showing promise as immune adjuvant for vaccines against intracellular infectious agents such as viruses, bacteria, protozoa and against cancer cells.

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