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# Immunomodulatory activity of biopolymeric fraction RLJ-NE-205 from *Picrorhiza kurroa*

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**Abstract**

In the last three decades, numerous biopolymeric fractions have been isolated from medicinal plants and used as a source of therapeutic agents. The most promising biopharmacological activities of these biopolymers are their immunomodulatory effects. The biopolymeric fraction RLJ-NE-205 was isolated and purified from the rhizomes of *Picrorhiza kurroa*. We evaluated the effects of biopolymeric fraction RLJ-NE-205 from *P. kurroa* on the in vivo immune function of the mouse. Balb/c mice were treated with the biopolymeric fraction RLJ-NE-205 (12.5, 25 and 50 mg/kg body weight) for 14 days with sheep red blood cells (SRBC) as an antigen. Haemagglutination antibody (HA) titre, plaque forming cell (PFC) assay, delayed type hypersensitivity (DTH) reaction, phagocytic index, proliferation of lymphocytes, analysis of cytokines in serum and CD4/CD8 population in spleen (determined by flowcytometry) were studied. At the dose of 50 mg/kg, significant increases in the proliferation of lymphocytes ($p<0.001$) and cytokine levels (IL-4 and IFN-gamma) in serum ($p<0.001$) were observed. A dose dependent increase was demonstrated in HA titre ($p<0.05$), DTH ($p<0.01$), PFC ($p<0.05$), phagocytic index ($p<0.05$) and CD4/CD8 ($p<0.01$) population. This suggests that the biopolymeric fraction RLJ-NE-205 improves the immune system and might be regarded as a biological response modifier.


*Keywords:* *Picrorhiza kurroa*; Haemagglutination antibody titre; Plaque forming cell assay; Delayed type hypersensitivity

## 1. Introduction

During the past 30 years, many biopolymeric fractions have been isolated from mushrooms, fungi, yeast, algae and lichens. More and more attention was cast on biopolymeric fractions isolated from plants and their various biological activities, especially immunostimulatory and adjuvant effects. Therefore, it is of interest to discover and evaluate biopolymeric fractions as new safe plant-based compounds with immunostimulatory activity [1,2].

Plant polysaccharides from Indian medicinal plants are considered to have immunomodulatory properties. However, systematic evaluations of in vivo immunomodulatory activities have been carried out for only a handful of these plants. The main source for the isolation of biopolymeric constituents, particularly oligo and polysaccharides, has been the organic solvent exhausted marc of *Picrorhiza kurroa*. The crude isolate has been evaluated for immunomodulatory activity. Most of the polysaccharides have been isolated from

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fungi and lichens [3,4]. Lentinan, a yeast polysaccharide isolated from *Lentinus edodes*, is one of the best-studied polysaccharides [5,6].

In an effort to search for new immunomodulators, the biopolymeric fraction RLJ-NE-205 from *P. kurroa* was screened for its immunostimulatory activity on the cellular and humoral immune response of Balb/c mice in vivo. We wish to report here the results of this study.

## 2. Materials and methods

### 2.1. Materials

The organic solvent exhausted material (0.5 kg) of the plant *P. kurroa* was used in this study. Trichloroacetic acid (TCA) and trifluoroacetic acid (TFA) were purchased from Merck (India). Medium RPMI 1640 (Himedia, Bombay, India), 96 V wells microtitration plates and microtissue culture plates (96 U wells) from Tarson, trypan blue (Microlabs, Bombay), fetal calf serum (FCS), Concanavalin-A (Con-A), lipopolysaccharide (LPS, *Escherichia coli* 055 B5), gum acacia, dimethyl sulphoxide (DMSO), Hank's balanced salt solution (HBSS), HEPES, 2-mercaptoethanol, penicillin, streptomycin, MTT (3-[(4,5-dimethylthiazol-2yl)-2,5-diphenyl 2,5-dimethyltetrazolium bromide), levamisole (lev) and cyclophosphamide (cyclo) (Sigma) were used. Fluoroisothiocyanate (FITC)-labeled CD4 anti-mouse monoclonal antibody, phycoerytherin (PE)-labeled CD8 anti-mouse monoclonal antibody, FACS lysing solution (BD Biosciences).

### 2.2. Preparation of biopolymeric fraction RLJ-NE-205 from P. kurroa

The organic solvent exhausted material (0.5 kg) of the plant *P. 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 extract was diluted with alcohol (1: 6) and kept overnight at 4 °C. The resultant precipitate was collected by 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 precipitate named as biopolymeric fraction RLJ-NE-205 from *P. kurroa* (3.5 g) obtained by centrifugation was suspended in warm distilled water (500 ml), and centrifuged. The aqueous solution was lyophilized. Biopolymeric fraction RLJ-NE-205 from *P. kurroa* (3.0 g) was obtained as an amorphous solid.

### 2.3. Hydrolysis of biopolymeric fraction RLJ-NE-205 from P. kurroa

Biopolymeric fraction (1.0 g) RLJ-NE-205 from *P. kurroa* was suspended in 50 ml of aqueous 2 M TFA and then refluxed (120 °C) for 2.5 h. The reaction mixture was concentrated under reduced pressure on a film evaporator and then kept in a desiccator containing NaOH, overnight. Paper chromatography of the hydrolysed biopolymeric fraction RLJ-NE-205 from *P. kurroa* in comparison with reference monosaccharides revealed the presence of arabinose, glucose, xylose and galactose.

### 2.4. Quantitative analysis of monosaccharides in the biopolymeric fraction RLJ-NE-205 from P. kurroa hydrolyzate by HPLC

HPLC grade water was prepared from Milli-Q water purification system. All the four monosaccharides i.e. D-glucose, D-xylose, D-galactose and D-arabinose were procured from Aldrich chemicals of purity ≤98% (HPLC).

### 2.5. Chromatography

Monosaccharides were separated and quantified by using a Shimadzu HPLC system consisting of Pump LC-10 AT*VP*, an automatic sampling unit (Autosampler), SIL-10 AD*VP*, a Column oven CTO-10 AS*VP*, *RI* detector and System controller SCL-10A*VP* version 5.4. Shimadzu Class *VP* software version 6.1 was used for data analysis and data processing. The samples were analyzed at 80 °C on a Phenomenex Rezex RPM-monosaccharide $Pb^{2+}$ (8%) column (300×7.80 mm) by RI detector using a gradient mobile phase of HPLC grade water.

### 2.6. Sample preparation

The accurately weighed quantity of the dried hydrolysate of biopolymeric fraction RLJ-NE-205 from *P. kurroa* was dissolved in a known volume of HPLC grade water. The samples were filtered through a millipore micro filter (0.45 μm) and then injected into the HPLC system.

### 2.7. Preparation of stock solutions and samples

Stock solutions of the pure reference compounds were prepared in HPLC grade water and stored in a refrigerator at 4 °C. From the stock solutions, working solutions for each reference compound were prepared by dilution with HPLC grade water. These working solutions of all the reference compounds were mixed together in equal volumes for further analysis.

### 2.8. Quantification

The compounds exhibited linear responses in the calibration curves, which were prepared by using the multipoint calibration curve method. Working solutions after mixing were injected in different amounts (2–20 μl). Excellent calibration curves were obtained for D-glucose, D-xylose, D-galactose and D-arabinose ($r^2$=1.0) in each case. Calibration curves were determined on the basis of six amounts (2–20 μl) of each standard in the mixture.

From HPLC of the hydrolysed (2 NTFA) biopolymeric fraction from *P. kurroa* in comparison to authentic monosaccharides it was observed that biopolymeric fraction RLJ-NE-205 is composed of glucose (27.8 min), xylose (30.1 min), galactose (33.1 min) and arabinose (36.9 min), in the molar ratio of 3.0, 1.0, 1.8 and 4.1.

### 2.9. Animals and treatment

#### 2.9.1. Animals

The study was conducted on male Balb/c mice (18–22 g). Male guinea pigs (250 g) were used for the preparation of complement for PFC assay. The ethical committee of the Regional Research Laboratory (CSIR) instituted for animal handling approved all protocols. The animals were bred and maintained under standard laboratory conditions: temperature (25±2 °C) and photoperiod of 12 h. Commercial pellet diet and water were given ad libitum.

#### 2.9.2. Treatment

SRBC collected in Alsever's solution, were washed three times in large volumes of pyrogen free 0.9% normal saline and adjusted to a concentration of $5\times10^9$ cells/ml for immunization and challenge.

Animals were divided into seven groups of ten animals each: (Group I) normal control, received normal saline; (Group II) vehicle control, received 1% gum acacia; (Group III) biopolymeric fraction RLJ-NE-205 (12.5 mg/kg body weight); (Group IV) biopolymeric fraction RLJ-NE-205 (25 mg/kg body weight); (Group V) biopolymeric fraction RLJ-NE-205 (50 mg/kg body weight); (Group VI) negative control, received cyclo (50 mg/kg body weight); (Group VII) positive control, received lev (2.5 mg/kg body weight).

Normal and vehicle control mice received normal saline and 1% gum acacia administered per oral (p.o.) while the negative as well as positive control mice received cyclo and lev also administered per oral. The biopolymeric fraction RLJ-NE-205 was dissolved in 1% gum acacia and was administered per oral for 14 days. The dose volume was 0.2 ml.

### 2.10. HA titre

The animals were immunized by injecting 0.2 ml of 10% of fresh SRBC suspension intraperitonially on day 0. Blood samples were collected in microcentrifuge tubes from individual animals by retro-orbital plexus on day 7 for primary antibody titre and day 15 for secondary antibody titre. Serum was separated and antibody levels were determined by the haemagglutination technique [7]. Briefly, equal volumes of individual serum samples of each group were pooled. Two fold dilutions of pooled serum samples were made in 25 μl volumes of normal saline in a microtitration plate to which were added 25 μl of 1% suspension of SRBC in saline. After mixing, the plates were incubated at room temperature for 1 h and examined for haemagglutination under the microscope. The reciprocal of the highest dilution of the test serum giving agglutination was taken as the antibody titre.

### 2.11. PFC assay

In brief, 0.2 ml of 10% SRBC prepared in normal saline was injected to animals, i.p. The animals were sacrificed on the fifth day of immunization. The spleen was removed, cleaned free of extraneous tissues and a single cell suspension of $10^6$ cells/ml was prepared from it in RPMI-1640 medium. For PFC assay, the SRBC were prepared at a cell density of $5\times10^8$ cells/ml in PBS. One milliliter of SRBC in medium along with 0.5 ml of diluted guinea pig complement (1 ml of serum+9 ml of normal saline) was added to 1 ml of spleen suspension. Cunningham chambers were prepared using a glass slide, coverslips and doubled-sided tape. The chambers were loaded with a known volume of assay mixture, sealed with xylene and incubated at 37 °C for 1 h. The plaques were counted under a light microscope and expressed as PFC per $10^6$ spleen cells [8].

### 2.12. DTH reaction

Biopolymeric fraction RLJ-NE-205 was administered 2 h after SRBC injection and once daily on consecutive days. Six days later, the thickness of the left hind footpad was measured with a spheromicrometer (pitch, 0.01 mm) and was considered as a control. The mice were then challenged by injecting 20 μl of $5\times10^9$ SRBC/ml intradermally into the left hind footpad. The foot thickness was measured again after 24 h [9].

### 2.13. Splenocyte proliferation assay

Spleen collected under aseptic conditions in HBSS was minced using a pair of scissors and passed through a fine steel mesh to obtain a homogeneous cell suspension and the erythrocytes were lysed with ammonium chloride (0.8%, w/v). After centrifugation (380×*g* at 4 °C for 10 min), the pelleted cells were washed three times with PBS and resuspended in complete medium [RPMI 1640 supplemented with 12 mM HEPES (pH 7.1), 0.05 mM 2-mercaptoethanol, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10% FCS]. Cell number was counted with a haemocytometer by the trypan blue dye exclusion technique. Cell viability exceeded 95% [10].

To evaluate the effect of biopolymeric fraction RLJ-NE-205 on the proliferation of splenic lymphocytes, spleen cell suspension ($1\times10^7$ cell/ml) was pipetted into 96 well plates (200 μl/well) and cultured at 37 °C for 72 h in a humid saturated atmosphere containing 5% $CO_2$ in the presence of Con-A (5 μg/ml) and LPS (10 μg/ml). After 72 h, 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. To each well, 100 μl of a DMSO working solution (192 μl DMSO with 8 μl 1 M HCl) was added, and the absorbance was evaluated in an ELISA reader at 570 nm with a 630 nm reference after 15 min.

### 2.14. Determination of IL-4 and IFN-gamma by the ELISA method

Serum was collected 4 h after final oral administration of the biopolymeric fraction RLJ-NE-205. The IFN-gamma and IL-4 were measured with an enzyme linked immunosorbent assay (ELISA kit, R&D Systems Quantikine) according to the instructions of the manufacturer [11,12].

### 2.15. Macrophage phagocytosis by carbon clearance method

The phagocytic clearance of the endothelial system was assayed in groups of ten mice each by injecting (i.v.) 160 mg/kg of 1.6% suspension of gelatin stabilized carbon particles [13]. Blood samples were collected before and at intervals varying between 2 and 90 min after carbon injection. An aliquot (10 μl) of the sample was lysed with 2 ml of 0.1% acetic acid and the transparency determined spectrophotometrically at 675 nm (Uvikon 810, spectrophotometer, Kontron Ltd., Switzerland) [14]. Biopolymeric fraction RLJ-NE-205 was administered orally for 7 days and 30 min prior to the carbon injection. The rate of carbon clearance termed as phagocytic index was calculated.

### 2.16. Lymphocyte phenotyping in spleen

The spleen (1/3 of the organ) was placed in PBS buffer (without $Mg^{2+}$ and $Ca^{2+}$) and stored on ice prior to preparation of single cell suspensions. Splenic erythrocytes were lysed with red blood cell lysing buffer (BD Pharmingen). Cell suspensions were refrigerated (ca. 4 °C) pending staining with antibodies. All reagents were purchased at BD Pharmingen. For each sample, $2 \times 10^6$ cells were stained with conjugated anti-CD4 FITC and anti-CD8a antibodies. After staining with antibodies, cells were washed and resuspended in PBS for flow cytometric analysis, which was performed on a FACS Calibur flow cytometer equipped with Cell Quest software (Becton Dickinson).

### 2.17. Statistical analysis

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

## 3. Results

### 3.1. HA titre

Biopolymeric fraction RLJ-NE-205 (12.5–50 mg/kg, p.o.) produced a dose-related increase in the primary and secondary antibody synthesis. The maximum effect was observed at 50 mg/kg (63.3% increase) in primary and (44.8% increase) in secondary antibody titre. Its effect was more significant in primary as compared to secondary antibody synthesis (Table 1). Administration of cyclo (50 mg/kg, p.o.) and lev (2.5 mg/kg, p.o.), used as negative and positive control respectively, resulted in a significant decrease and increase in humoral antibody titre compared with the control animals (Group I).

### 3.2. PFC assay

The effect of biopolymeric fraction RLJ-NE-205 on the number of PFC in spleen is shown in Fig. 1. The maximum increase in the PFC assay in biopolymeric fraction RLJ-NE-205 treated groups at 50 mg/kg was observed on the 5th day after the immunization in comparison with control animals. Cyclo and lev as negative and positive controls produced significant inhibition and stimulation in the PFC assay.

### 3.3. DTH reaction

DTH reaction to SRBC is shown in Table 2, in which data are expressed in terms of the swelling of the footpad.

Table 1
Effect of biopolymeric fraction RLJ-NE-205 from Picrorhiza kurroa on humoral antibody (HA) titre and macrophage phagocytosis

| Treatment | Doses (mg/kg) | Humoral immune response | | Phagocytic index (mean±S.E.M.) |
|---|---|---|---|---|
| | | Primary Ab titre 7th day, mean±S.E.M. (% Change) | Secondary Ab titre 15th day, mean±S.E.M. (% Change) | |
| Control | – | 6.00±0.44 | 5.80±0.20 | 0.083±0.04 |
| Vehicle control | – | 6.20±0.24 (3.3↑) | 6.00±0.44 (3.3↑) | 0.091±0.03 (9.6↑) |
| Lev | 2.5 | 8.4±0.24*** (40.0 ↑) | 7.60±0.44** (31.0 ↑) | 0.152±0.02** (83.1 ↑) |
| Cyclo | 50 | 5.2±0.24 (13.3 ↓) | 4±0.00 (31.0 ↓) | 0.061±0.02 (26.5 ↓) |
| RLJ-NE-205 | 12.5 | 7.4±0.24 (23.3 ↑) | 7.00±0.44 (20.6 ↑) | 0.09±0.01 (8.4 ↑) |
| RLJ-NE-205 | 25 | 8.20±0.20* (36.6↑) | 7.8±0.37* (34.4 ↑) | 0.137±0.04* (65.0 ↑) |
| RLJ-NE-205 | 50 | 9.8±0.20** (63.3 ↑) | 8.40±0.24** (44.8 ↑) | 0.176±0.04** (112.0 ↑) |

[a]HA titres were determined on day 7 for primary and day 15 for secondary antibody titre; (↑) stimulation, (↓) suppression.
[b]Phagocytic index represents the average number of bacteria or inert particles ingested per phagocytic cell during a given time period.
[c]Numbers of mice are 10 for each group. The differences between the control and biopolymeric fraction treated groups were determined by one-way Anova (Bonferroni correction multiple comparison test). Data are expressed as Mean±S.E.M. *$P<0.05$; **$P<0.01$ and ***$P<0.001$ compared with the control group.

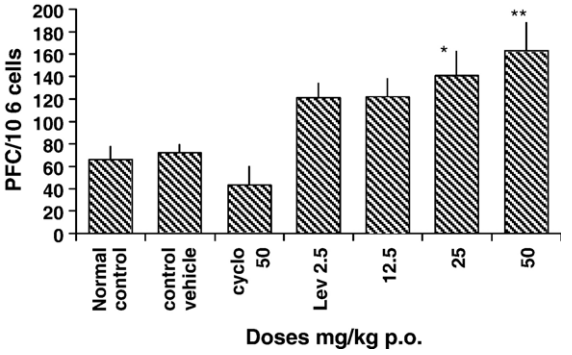


Fig. 1. Effect of biopolymeric fraction RLJ-NE-205 from *P. kurroa* (12.5–50 mg/kg) on humoral immunity as assessed by PFC assay. Data are means ± S.E.M. of ten animals. *$p<0.05$ and **$p<0.01$ when compared with control group determined by one-way ANOVA (Bonferroni correction multiple comparison test).

Administration of the biopolymeric fraction RLJ-NE-205 (12.5–50 mg/kg) produced a significant increase in the DTH reaction compared to control (Group I).

### 3.4. Splenocyte proliferation assay

To understand the immunomodulatory activity of biopolymeric fraction RLJ-NE-205 from *P. kurroa*, we investigated its effects on the proliferation of spleenic cells. All of the test doses (12.5–50 mg/kg) significantly increased the proliferation of spleenic cells in a dose dependent manner (Fig. 2). The Con-A (5 μg/ml) and LPS (10 μg/ml) stimulated spleenocyte proliferation was significantly enhanced by biopolymeric fraction RLJ-NE-205 at 50 mg/kg and cellular proliferation was increased up to threefold in Con-A and twofold in LPS treated cells, respectively, compared to the control (Group I).

Table 2
Effect of biopolymeric fraction RLJ-NE-205 from *P. kurroa* on SRBC-induced DTH response (CM1) in mice

| Treatment | Doses (mg/kg) | Cell mediated immune response Paw swelling (mm) in mice day 7 | |
|---|---|---|---|
| | | Mean ± S.E.M. | (% Change) |
| Control | | 0.81 ± 0.04 | |
| Vehicle control | | 0.86 ± 0.03 | 6.1 ↑ |
| Lev | 2.5 | 1.24 ± 0.16* | 53.0 ↑ |
| Cyclo | 50 | 0.69 ± 0.03 | 14.8 ↓ |
| RLJ-NE-205 | 12.5 | 1.14 ± 0.12* | 40.7 ↑ |
| RLJ-NE-205 | 25 | 1.26 ± 0.13 | 55.5 ↑ |
| RLJ-NE-205 | 50 | 1.48 ± 0.22** | 82.7 ↑ |

[a]Numbers of mice are 10 for each group. (↑) Stimulation, (↓) suppression.
The differences between the control and biopolymeric fraction treated groups were determined by one-way ANOVA (Bonferroni correction multiple comparison test). Data are expressed as Mean ± S.E.M. *$P<0.05$ and **$P<0.01$ compared with the control group.

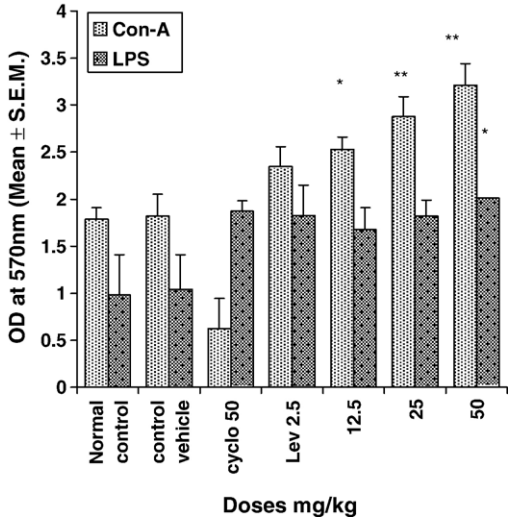


Fig. 2. Effect of biopolymeric fraction RLJ-NE-205 from *P. kurroa* (12.5–50 mg/kg) on splenocyte proliferation. Proliferation activity was expressed as the absorption at 570 nm. Values are means ± S.E.M. of ten mice; **$p<0.01$ and *$p<0.05$ when compared with control group determined by one-way ANOVA (Bonferroni correction multiple comparison test).

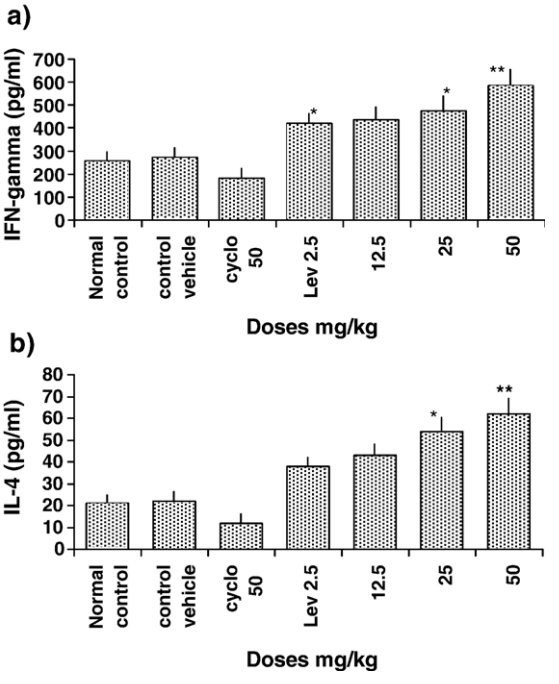


Fig. 3. Concentration of IFN-gamma and IL-4 in mouse serum. The IFN-gamma and IL-4 concentration in pg/ml were determined using ELISA. Values are means ± S.E.M. of ten mice; *$p<0.05$ and **$p<0.01$ when compared with control group determined by one-way ANOVA (Bonferroni correction multiple comparison test).

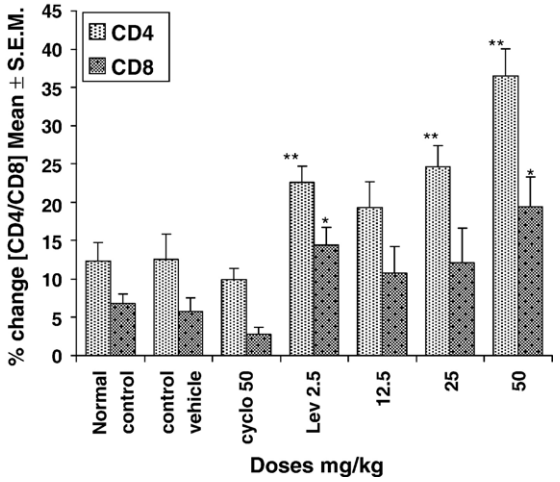


Fig. 4. Flowcytometric analysis of T cell surface antigen CD4 (FITC-conjugated monoclonal antibody) and CD8 (PE-conjugated monoclonal antibody), day 14. Values are means±S.E.M. of ten mice; $^{**}p<0.01$ and $^{*}p<0.05$ when compared with control group determined by one-way ANOVA (Bonferroni correction multiple comparison test).

### 3.5. Concentration of IFN-γ and IL-4

At doses of 12.5, 25 and 50 mg/kg, fraction RLJ-NE-205 produced a significant increase in the levels of IFN-γ and IL-4 compared with the control group (Fig. 3).

### 3.6. Macrophage phagocytosis by carbon clearance method

The effects of biopolymeric fraction RLJ-NE-205 from *P. kurroa* on the phagocytic function of macrophages are shown in Table 1. Compared with the control group, 50 mg/kg of the fraction RLJ-NE-205 caused a significant increase in the phagocytic index, but no significant increase was observed for the dosages of 12.5 and 25 mg/kg.

### 3.7. Lymphocyte phenotyping in spleen

The effect of biopolymeric fraction RLJ-NE-205 on $CD4^+$ and $CD8^+$ T cells lymphocytes is shown in Fig. 4. Compared with the control group, 25 mg/kg and 50 mg/kg of the fraction caused a significant increase of $CD4^+$ and $CD8^+$ T cells lymphocytes, but no significant increase was observed for the dosage of 12.5 mg/kg. Cyclo (50 mg/kg, p.o.) and lev (2.5 mg/kg, p.o.) inhibited and stimulated both $CD4^+$ and $CD8^+$ T cells, respectively.

## 4. Discussion

The prime objective of the study was to investigate the immunomodulatory effect of biopolymeric fraction RLJ-NE-205 from *P. kurroa.* There is a growing interest in identifying plant-based immunomodulators ever since their possible use in modern medicine was suggested [15,16]. The findings outlined above have demonstrated that biopolymeric fraction RLJ-NE-205 from *P. kurroa* possesses a potent immunostimulant action.

The immune response of the body is mainly composed of specific and non-specific immunity. The specific immune response includes humoral and cellular immunity. Humoral immunity, via the antibody response, is regulated by B cells and other immune cells involved in antibody production. The stimulation of the humoral response against SRBCs by biopolymeric fraction RLJ-NE-205 was evidenced by the increase in HA titer and PFC assay in mice. The fraction also led to an increase in the serum levels of the cytokine IL-4 and might enhance the humoral immune response by this mechanism.

In our present investigation, we found that biopolymeric fraction RLJ-NE-205 could augment Con-A and LPS induced splenocyte proliferation. A DTH reaction is an expression of cell-mediated immunity and plays a role in many inflammatory disorders [17]. Such reactions are characterized by large influxes of non-specific inflammatory cells, of which the macrophage is a major example. It is a type IV hypersensitivity reaction that develops when antigen activates sensitized $T_{DTH}$ cells. These cells generally appear to be a $T_H1$ subpopulation although sometimes $T_C$ cells are also involved. Activation of $T_{DTH}$ cells by antigen presented through appropriate antigen presenting cells results in the secretion of various cytokines includes interferon-gamma (secreted by $T_H1$ cells). The overall effects of these cytokine are to recruit and activate macrophages, thereby promoting increased phagocytic activity. Several lines of evidence suggest that DTH reactions are important in host defense against parasites and bacteria that can live and proliferate intracellularly. Treatment with biopolymeric fraction RLJ-NE-205 enhanced the DTH reaction, as reflected by the increased footpad thickness compared to the control group, suggesting heightened infiltration of macrophages to the inflammatory site. This study may support a possible role of biopolymeric fraction RLJ-NE-205 in assisting the cell-mediated immune response.

In view of the pivotal role played by macrophages in coordinating the processing and presentation of antigen to B-cells, biopolymeric fraction RLJ-NE-205 was evaluated for its effect on macrophage phagocytic activity. Higher doses of the fraction were found to increase the phagocytic index as assessed by the clearance of intravenous carbon particles.

The biopolymeric fraction RLJ-NE-205 had a significant stimulatory effect on $CD4^+$ and $CD8^+$ T cells, thereby confirming its general effect on the cell-mediated immune response.

In this study, we found that biopolymeric fraction RLJ-NE-205 from *P. kurroa* could significantly increase the phagocytic function of macrophages, the proliferation of Con-A and LPS stimulated spleenic lymphocytes, the CD4/CD8 population in spleen, HA titre, PFC assay, the DTH reaction and cytokines levels (IL-4 and IFN-γ). This suggests that the fraction RLJ-NE-205 may enhance non-specific, humoral and cellular immunity in a mouse model. Acute toxicological studies with biopolymeric fraction RLJ-NE-205 were determined by oral administration in mice where the $LD_{50}$ was found to be greater than 1800 mg/kg.

In conclusion, the present study has shown the immunostimulatory activity of biopolymeric fraction RLJ-NE-205 from *P. kurroa* and suggests its therapeutic usefulness. Biopolymeric fraction from *P. kurroa* stimulated both the humoral and cellular arms of the immune system. Further detailed studies are required which might establish a possible use in immunocompromised animals and as an adjuvant during vaccination programs in order to reduce the number of non-responders to vaccines. However, detailed studies of mechanisms of immunomodulation and probable use in immunocompromised individuals are still to be investigated.

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