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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.