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Immunomodulatory activity of ether insoluble phenolic components of *n*-butanol fraction (EPC-BF) of flaxseed in rat

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ABSTRACT

Objective: To investigate the immunomodulatory activity of ether insoluble phenolic components of *n*-butanol fraction (EPC-BF) of flaxseed in rat. **Methods:** Immunomodulatory activity of EPC-BF was assessed in rat at oral doses of 150 and 300 mg/kg using neutrophil adhesion test, heamagglutination test and measuring delayed type hypersensitivity response. **Results:** EPC-BF modulates immune response by significantly reducing cell-mediated immune response at oral dose 150 mg/kg but not at dose 300 mg/kg. However, EPC-BF did not affect on the neutrophil adhesion and humoral immunity at both the studied doses. **Conclusion:** EPC-PC modulates immune response by reducing cell-mediated immune response at lower dose but not at higher dose, which could be due to the presence of both immunostimulant and immunosuppressant phenols together in EPC-BF. These studies suggest that EPC-BF could be a promising source for new immune modifier.

1. Introduction

Searching of substances with immunostimulative or immunorestorative effects could contribute to the maintenance of the immune system[1]. Many plants have been evaluated for immunostimulant and immunosuppressive properties using simple techniques[2]. There are several plants such as *Tanacetum vulgare* (*T. vulgare*) L, *Actinidia macrosperma* (*A. macrosperma*), *Tinospora cordifolia* (*T. cordifolia*), *Curcuma longa* (*C. longa*), *Vernonia amygdalina* (*V. amygdalina*) *etc*. reported to have immunomodulatory properties[3-7]. Immune modulation helps to maintain disease-free state. Stimulation and suppression of immune response are the two ways of immunomodulation. Stimulation of immune response can be active, in which an immune response is induced through exposure to an antigen, or passive, in which preformed antibodies are administered directly. Most of the immune suppressive drugs, suppress immune response by inhibiting T cell activation, are used to prevent acute rejection after organ transplantation and to treat certain autoimmune diseases[8].

Linum usitatissimum (*L. usitatissimum*) L. belongs to family linaceae, commonly known as flaxseed or linseed. Flaxseed has been playing a major role in the field of diet and disease research due to its high content of omega-3 fatty acid, α-linolenic acid and a major lignan secoisolariciresinol diglucoside (SDG)[9]. Flaxseed or flaxseed oil is a well known immunomoduator. It has been reported that supplementation of flaxseed diet to human tend to suppress the cell-mediated immunity without affecting the humoral immunity in human[10]. Similarly, feeding of α-linolenic acid from flaxseed to rat also did not affect on antibody production[11]. However, role of flaxseed phenolic components in immunomodulation is still remains indistinct.

In our previous study, we have selectively isolated ether insoluble phenolic components (EPC-BF) such as caffeic acid, tannins and phenolic glycosides free from earlier reported ether soluble phenolics flavonoids and lignan, SDG from *n*-butanol fraction of defatted flaxseed meal and studied their antioxidant potential in various models[12,13]. The present study was undertaken to evaluate the effect of EPC-BF on immunomodulation in rat.

2. Materials and methods

2.1. Chemicals

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Hexane, methanol, n-butanol, solvent ether, sodium chloride, gum acacia were purchased from Molychem (Mumbai, India). Anesthetic ether (I.P.) was purchased from Narsons phaema, India. Alsever's solution was purchased from Sigma (St. Louis, MO, USA). All chemical and reagents were of analytical grade.

2.2. Preparation of extract

EPC-BF was prepared by our earlier descried method[13]. Briefly, a double pressed flaxseed cake powder obtained from 'Omega-3 oil unit, established under project NAIP-ICAR-Component-3 at Sangamner, M.S., India' was used for extraction. Residual oil from flaxseed meal was removed by *n*-hexane (1:4, w/v). Dried defatted flaxseed powder was extracted with methanol for 3 h at 60 ℃ in a soxhlet apparatus. Methanol extract was concentrated in rotavapour at 60 ℃ under vacuum. Then the dried residue was further partitioned with *n*-butanol: water (1:1, v/v). *n*-Butanol fraction was separated from aqueous fraction and dried in a rotavapour at 80 ℃ under vacuum. Dried *n*-butanol residue was dissolved in minimum quantity of methanol and precipitated with solvent ether (1:5, v/v), finally brown colored sticky precipitate of EPC-BF was obtained. Finally, it was dissolved in 2% gum acacia and used for the evaluation of immunomodulatory activity.

2.3. Animals

Female Wistar rats 120-200 g and 12 mice 25-35 g of either sex were used for the study. They were housed in animal house under standard condition of temperature (25 ± 2) ℃, 12/12 h light/dark cycle and fed with standard pellet diet (Amrut, Sangali, M.S., India) and tap water ad libitum. Institutional Animal Ethical committee, Amrutvahini College of Pharmacy, Sangamner approved the study protocol. The animals were divided into three groups consisting six animals each. Group I was of normal control received only vehicle i.e. 2% gum acacia. Animals of Group II and III received orally 150 and 300 mg/kg of EPC-BF (dissolved in 2% gum acacia) respectively.

2.4. Acute oral toxicity study

Adult Swiss albino female mice 18-22 g were subjected to acute oral toxicity studies as per guideline (AOT No. 425) suggested by Organization for Economic Co-operation and Development (OECD) (2001). Dose of 175, 550, 1 750, 2 000 and 5 000 mg/kg of EPC-BF were administered orally to mice. The mice were observed by housing them individually in the polypropylene metabolic cages continuously for 2 h for behavioral, neurological, autonomic profiles and for any lethality during next 48 h.

2.5. Preparation of antigen

Antigen was prepared by collecting sheep blood in sterile Alsever's solution in 1:1 proportion and kept in the refrigerator. Sheep Red Blood Cells (SRBCs) for immunization were prepared by centrifuging at 2 000 rpm for 10 min and washed 4-5 times with physiological saline and then suspended into buffered saline for desired cell concentration[14].

2.6. Immunomodulatory activity

2.6.1. Neutrophil adhesion test

Neutrophil adhesion test was carried out according to method described by Dashputre and Naikwade[15] with some modification. Rats were divided into three groups and treated orally with vehicle or EPC-BF for 14 d. On the 14th day of drug treatment, blood samples were collected from retro-orbital plexus into heparinized vials and analyzed for total leukocyte count (TLC) and differential leukocyte count (DLC) by the using hematology analyzer (Abacus Junior, Diatron, Australia). After initial counts, blood samples were incubated with 80 mg/mL of nylon fibers for 15 min at 37 ℃. The incubated blood samples were again analyzed for TLC and DLC. The product of TLC and percentage of neutrophil gives the neutrophil index (NI) of blood sample. Percentage of neutrophil adherence was calculated by equation:

$$\text{Percent neutrophil adhesion} = \frac{\text{NIu} - \text{NIt}}{\text{NIu}}$$

Where, NIu was neutrophil index of untreated blood sample and NIt neutrophil index of treated blood sample.

2.6.2. Delayed type hypersensitivity response (DTH response)

C ell mediated immune response was measured by measuring delayed type hypersensitivity response[16]. All rats were immunized on day 0 by i.p. administration of 5×10^9 SRBC/rat. The animals were treated with EPC-BF for 14 more days and challenged by subcutaneous administration of 1.25×10^9 SRBC/mL in to right hind foot pad on day 14. DTH responses were measured by using vernier caliper at 24 h after SRBCs challenged on day 14. The difference between pre and post challenge foot thickness expressed in cm was taken as a measure of delayed type hypersensitivity.

2.6.3. Determination of humoral immune response

Indirect haemagglutination test with some modification was used to measure humoral immune response[17]. Rats were pretreated with the EPC-BF for 14 d and each rat was immunized with 0.4 mL of 5×10^9 SRBC/rat by i.p. route, including control rats. The day of immunization was referred to as day 0. The treatment of EPC-BF was continued for another 14 d and blood samples were collected from individual rats from retro-orbital plexuses on 15th day for determination of Haemagglutinating Antibody (HA) titer. The titer value was determined by titrating serum dilution with SRBC (1.25×10^9 cells) in microtiter plate. The plate were incubated at room temperature for 2 h and examined visually for agglutination. The highest number of serum dilution showing haemagglutination was noted and expressed as HA titer.

2.7. Statistical analysis

Data were expressed as the mean ± S.E.M. Statistical analysis was carried out by one-way ANOVA followed by Dunnette comparison test using graphpad prism 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. $P<0.05$ was considered significant.