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# Effect of fenugreek (*Trigonella foenum-graecum* L.) galactomannan fractions on phagocytosis in rat macrophages and on proliferation and IgM secretion in HB4C5 cells

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**Abstract**

Fenugreek galactomannan rich fractions were tested for activation of phagocytosis in rat peritoneal macrophages, proliferation and IgM secretion in human–human hybridoma, HB4C5 cells. Alkali extracted polysaccharide B fraction showed 40% activation of phagocytosis in rat peritoneal macrophages at 10 μg/ml concentration. Fraction 1 of this extract showed an activity of 31.4% at 1 μg/ml in 1 h. In HB4C5 cells, growth promotion activities (%) expressed by alkali extracted polysaccharide B, Fraction 1 and Fraction 2 were 2.7 (at 5 μg/ml), 5.7 (at 2.5 μg/ml) and 5.6 (at 5 μg/ml), respectively. Effect on IgM secretion by alkali extracted polysaccharide B was more inhibitory with an inhibition of 24.0% at 2.5 μg/ml. Fraction 1 showed an activation of 9.8% at 5 μg/ml. At 2.5 μg/ml, it expressed an inhibition of 2.5%. Fraction 2 also expressed an inhibition of 24.7% at 2.5 μg/ml. 

*Keywords*: Fenugreek galactomannan; Macrophages; Phagocytosis; HB4C5 cells; Proliferation; IgM

## 1. Introduction

Fenugreek (*Trigonella Foenum-graecum* L.), an annual herb of leguminoseae, is being used as spice with its seeds and as vegetable with its plant (leaves). Though fenugreek is indigenous to Western Asia and Southern Europe, now it is mainly cultivated in India, Pakistan, France, Argentina and North African countries (Shankaracharya & Natarajan, 1972). Fenugreek is known for its various traditional medicinal purposes. Its use as aphrodisiac by Egyptians, and along with honey, in treatment of rickets, diabetes, dyspepsia, TB, rheumatism, anemia and constipation; as expectorant in veterinary practice are recorded (Charalambous, 1994). Its potential benefit in modulating carbohydrate (controlling plasma glucose) and lipid metabolism has been found by alteration of starch digestion and bile acid absorption (Madar, 1984). Some allergic reactions due to inhalation of fenugreek powder or due to application of its paste to scalp have been reported (Shlosberg & Egyed, 1983).

Reports on the effect of fenugreek galactomannan on immunological aspects are not available. Macrophages play an important role in cell mediated immunity by producing various kinds of cytokines like interleukin, interferon, tumor necrosis factor (TNF), active substances like prostaglandin, $H_2O_2$, super oxide and nitrite (Pick & Keisari, 1981). The measurement of phagocytic activity is a useful tool in the assessment of macrophage function (Kaminski, Roberts, & Guthrie, 1985). In the diagnosis and therapy of human diseases, human-monoclonal antibodies are being preferred over other types in view of their low antigenicity (Olsson & Mathe, 1982; Ritz & Scholossman, 1982). In this direction, hybridomas, producing human-monoclonal antibodies specific to various human antigens, are getting more importance. HB4C5 cells are the human–human hybridomas producing monoclonal antibodies against human lung cancer (Murakami, 1989; Murakami et al., 1985). This hybridoma is being used widely in screening of immunoglobulin production stimulating factor in food stuffs (Yamada, Ikeda, Sugahara, Shirahata, & Murakami, 1989a; Yamada, Ikeda, Maeda, Shirahata, & Murakami, 1990).

The present investigation deals with the effect of fenugreek galactomannan fractions on phagocytosis in rat peritoneal macrophages and on proliferation and IgM secretion in human–human hybridoma, HB4C5 cells.

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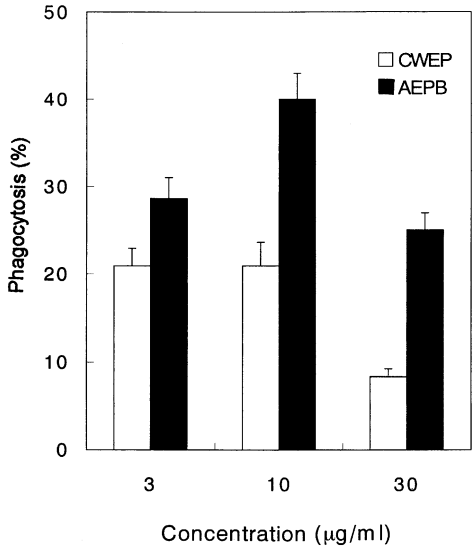


Fig. 1. Effect of CWEP and AEPB on phagocytosis in rat peritoneal macrophages. Peritoneal macrophages ($1 \times 10^5$ cells/well) cultured for 24 h were incubated with 2 μl of polysaccharide solution containing CWEP or AEPB at concentrations of 3, 10 and 30 μg/ml. Phagocytic activity was measured using FITC zymosan.

## 2. Materials and methods

### 2.1. Materials

Fluorescein isothiocyanate (FITC) zymosan from Molecular probes, USA; lipopolysaccharide (LPS) from Sigma, USA; 2,2′-azinodi-(3-ethylbenzthiazolin sulfonic acid (ABTS) and soluble starch from Wako Pure Chemical Co. Ltd, Japan; bactopeptone from DIFCO labs; Affinity Isolated Goat F (ab′)2 Anti-(Human IgM μ chain) peroxidase conjugate and Affinity Isolated goat antibodies Anti-(Human IgM μ chain) unconjugated from Biosource International; 1-methoxy-5-methylphenazinium methyl sulfate (1-Methoxy PMS) and water soluble tetrazolium (WST-1) from Dojindo; eRDF (enriched RDF, mixture of mediums viz. RPMI 1640, Dulbecco's MEM and Ham's F12) powder, RPMI 1640 from GIBCO, USA; Dulbecco's phosphate buffer saline (PBS(−)) from Nissui Pharmaceuticals, Japan; Fetal Bovine Serum from Filtron Co., Australia; Penicillin G potassium and streptomycin sulfate from Meiji Seika Japan.

Fenugreek galactomannan fractions viz. cold water extracted polysaccharide (CWEP), alkali extracted polysaccharide B (AEPB) and it's fractions Fra. 1 and Fra. 2, prepared as per Ramesh, Yamaki, Ono, and Tsushida (2001) were used in this study.

### 2.2. Methods

#### 2.2.1. Rat peritoneal macrophage activation (Ragsdale & Grasso, 1989)

Macrophages harvested from male albino rats (Sprague Dawley) obtained from Charles River Japan Inc., were used. Soluble starch and bactopeptone in physiological saline were injected (5 ml/100 g body weight) to peritoneum of the animals. On day 4, peritoneal macrophages were harvested in Hank's balanced salt solution (HBSS). After washing (×3), cells in RPMI 1640 {supplemented with fetal calf serum (FCS) and antibiotics, penicillin and streptomycin} were plated at a cell density of $1 \times 10^5$ cells/well. Incubation was done at 37 °C with 5% $CO_2$ for 2 h. Cells were washed (×3) with RPMI 1640 and cultured for 24 h. 2 μl of samples with known concentrations were added to the test wells. After 1 h incubation, 20 μl FITC zymosan was added and again incubated for 1/2–1 h. After washing (×3) with HBSS, 100 μl of triton x-100 (10%) was added. Fluorescence measurements were done in a Cytofluor 2350 (Millipore) with PS/V accelerator at an excitation λ of 485 nm and an emission λ of 530 nm.

#### 2.2.2. Proliferation and IgM secretion in HB4C5 cells

HB4C5 cells were cultured in a serum-free eRDF-ITES (insulin, transferrin, ethanolamine and sodium selenite) medium. Cells at log phase were collected by centrifugation at 1000 rpm and washed with PBS. Cells in eRDF-ITES medium were plated in 96 well micro-plates at a cell density of $2 \times 10^4$ cells/ml for proliferation studies or $1 \times 10^5$ cells/ml for IgM secretion studies. Known concentration of samples were added and cells were cultured for 4 days at 37 °C in a humidified 5% $CO_2$ and 95% air atmosphere. On the 5th day, cell growth was studied by proliferation assay (Shinmoto, Miyama, Kobori, & Tsushida, 1996) using WST-1 reagent and OD was read at 450 nm in a microplate reader. IgM in the culture supernatants was determined by ELISA (enzyme linked immunosorbent assay) (Shinmoto, Murakami, Dosako, Shinohara, & Omura, 1986). The absorbance was measured in an ELISA reader (Molecular Devices, WAKO) at 405 nm.

## 3. Results and discussion

Galactomannans from different sources and having structural variations in anomeric configurations; galactose and mannose ratio and also in galactose substitution patterns, have been found to possess different biological activity. A galactomannan from Aspergillacea cell wall has been detected immunohistochemically in the cytoplasm of phagocytic cells during invasive aspergillosis (Pierard, Estrada, Franchimont, Thiry, & Stynen, 1991). A branched galactomannan {(1 → 6)-linked α-D-mannopyrannosyl and α-D-(1-6)-galactopyrannosyl} from Iceland moss, *Cetraria islandica* (L.) Ach., has been found in vitro to possess pronounced immunostimulating activity (Ingolfsdottir, Jurcic, Fisher, & Wagner, 1994). In the present investigation with fenugreek galactomannan, AEPB fraction showed more phagocytic activating property as compared to CWEP (Fig. 1). The minimum activity expressed by AEPB was 25.0% at 30 μg/ml concentration and the

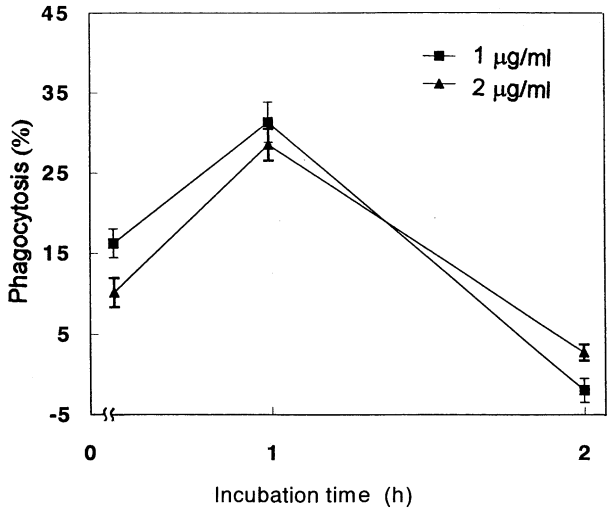


Fig. 2. Effect of Fra. 1 on phagocytosis in rat peritoneal macrophages. Peritoneal macrophages ($1 \times 10^5$ cells/well) cultured for 24 h were incubated with 2 μl of polysaccharide solution containing Fra. 1 at concentrations of 1 and 2 μg/ml. Phagocytic activity was measured using FITC zymosan.

maximum was 40.0% at 10 μg/ml. CWEP showed a minimum activation of 8.3% at 30 μg/ml concentration and maximum activity was 21.0% at 3 μg/ml. As shown in Fig. 2, an activity of 31.4%, in 1 h at 1 μg/ml, was expressed by Fra. 1, derived from AEPB. The percentage activation has been expressed with respect to the base control value of PBS. The differences in activation of phagocytosis expressed by these fractions could be attributed to the purity of the respective fraction.

Human–human hybridoma, HB4C5 cells are the hybridomas produced by fusing NAT-30 derived from human B lymphoma cell line Namalwa, with B lymphocytes of lymph node from a lung large cell carcinoma patient (Murakami et al., 1985). Fig. 3 shows the effect of AEPB and its purified fractions 1 and 2 on the growth of HB4C5 cells. AEPB showed a minimum activity of 1.0% at 2.5 μg/ml concentration and a maximum activity of 26.8% at 5 μg/ml. Its fraction, Fra. 1 showed a maximum activity of 5.7% at 2.5 μg/ml and a minimum activity of 0.37% at 10 μg/ml. However, Fra. 2 showed a maximum activity of 5.6% at 5 μg/ml and a minimum activity of 1.1% at 2.5 μg/ml. Individually, though these fractions slightly induced the growth, together they seem to be inhibitory in their effect. The extent of proliferation stimulating activity of various food components have been found also to depend on cell density at which it was tested. It may exhibit higher activity at lower cell densities and lower activity at higher cell densities as observed with lactoferrin, egg yolk lipoprotein, Royal jelly and Block Ace (Glicksman, 1982).

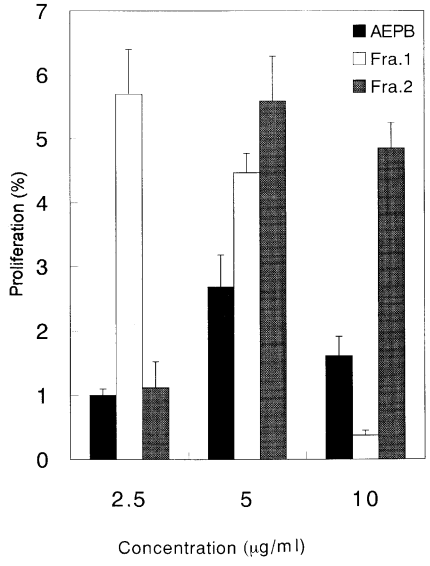


Fig. 3. Effect of different fractions on proliferation in HB4C5 cells. HB4C5 cells ($2 \times 10^4$ cells/ml) were cultured for 4 days in the presence of polysaccharide fractions viz. AEPB, Fra. 1 and Fra. 2 at concentrations of 2.5, 5 and 10 μg/ml. On the 5th day, proliferation was assayed using WST-1 reagent.

Various food stuffs/components have been screened for IgM production stimulating activity using HB4C5 hybridoma cells (Fuke, Ooishi, & Shinohara, 1994; Yamada et al., 1990). In the present investigation, according to Fig. 4, AEPB showed a maximum inhibition of 24.0% of IgM production at 2.5 μg/ml concentration and a minimum of 9.8% at 5 μg/ml. Fra. 2 also showed more or less similar pattern of inhibition, 24.7% at 2.5 μg/ml and 12.0% at 5 μg/ml concentration. However, the minimum inhibition observed by this fraction was 0.84% at 10 μg/ml. Effect of Fra. 1 was found to be different. In that, though it inhibited IgM secretion to an extent of 2.5% at 2.5 μg/ml, at the other concentrations it activated the secretion. Maximum activation of 9.8% was observed at 5 μg/ml and minimum activation of 5.5% was found at 10 μg/ml concentrations.

There are many reports about the immunomodulatory effects of cell wall galactomannans (Blake, Dhal, Herron, & Nelson, 1991; Grando, Hostager, Herron, Dhal, & Nelson, 1992). The property of repetitive moieties or subunits that usually occur in these polysaccharides resulting in identical epitopes has been reported to be involved in immunogenicity (Paraf & Peltre, 1991). Some polysaccharides have been found to be immunoglobulin production stimulating factors (Yamada, Ikeda, Sugahara, Shirahata, & Murakami, 1989b; Yamada et al., 1990). Soya been hull hemicellulose has been found to stimulate IgM production in HB4C5 hybridoma and lymphocytes (Maeda, Yamada, Ohta, Tajima, & Murakami, 1991b). The extent of stimulation of IgM production has been found related also to cell densities. Lactoferrin, egg yolk lipoprotein, Royal jelly, Block Ace all have been found to significantly stimulate IgM production in HB4C5 hybridoma at lower cell densities where as at higher cell densities their stimulatory effect was found to be little (Yamada et al., 1989a). Considering the effects of AEPB and its fractions on both proliferation and IgM secretion and with the exception of activation of IgM secretion found by Fra. 1 at 5 and 10 μg/ml, it looks that

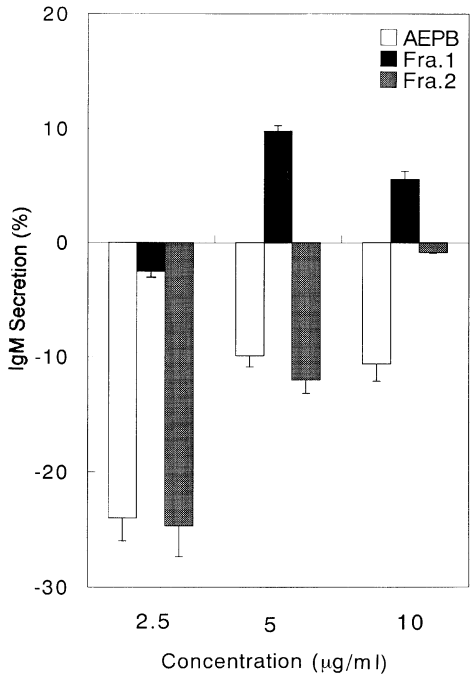


Fig. 4. Effect of different fractions on IgM secretion in HB4C5 cells. HB4C5 cells ($1 \times 10^5$ cells/ml) were cultured for 4 days in the presence of polysaccharide fractions viz. AEPB, Fra. 1 and Fra. 2 at concentrations of 2.5, 5 and 10 μg/ml. On the 5th day, IgM was determined by ELISA.

whenever there is slight activation of proliferation, the effect on IgM production is inhibitory.

Mannan from cell walls have been found to inhibit immune response (Nelson, Shibata, Podzorski, & Herron, 1991). Regarding molecular weight considerations upon the activation or inhibition, even the very high molecular weight species have been found to express their activity. Two polysaccharide fractions of molecular weights more than 100 KD from dried safflower petals induced IgM production in vitro in spleen cells derived from C57BL/6 mice (Wakabayashi, Kataoka, Woo Je-Tae, & Nagai, 1998). Dextran sulfate, xylan, curdlan, arabinogalactan and glucomannan have been found to be poor stimulators of IgM production (Maeda, Tajima, Yamada, & Murakami, 1991a). Locust bean gum, pectin, soybean hull hemicellulose and chitin are found to stimulate IgM production (Maeda, Mori, Tajima, & Murakami, 1992). Locust bean galactomannan has a gal/man content of 1:4 (Grasdalen & Painter, 1980). The functional differences of galactomannan fractions observed in the present investigation, in activation of phagocytosis, proliferation and IgM secretion, may be attribted to the differences in their structure. Both CWEP and AEPB contain some traces of glc where as Fra. 1 and Fra. 2, derived from AEPB, have a gal/man content of 1:1.04 and 1:1.12, respectively, and they may also differ in their chain length/molecular weight (as reflected by GPC (Ramesh et al., 2001). Regarding inhibition of IgM secretion in HB4C5 cells, more experimental data at different cell densities may lead to an understanding of this function.

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