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Hypercholesterolemic and Immunomodulatory Effects of Oat Extracts containing β -glucan

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Abstract: An experimental research was carried out to study the effect of oat extracts as modulators of immune response and hypercholesterolemia. This research involved experiments to examine the influence of oat extracts on blood lipids in mice. Oat extracts were given in intraperitoneally to Swiss albino mice fed on hypercholesterolemic diet or normal diet. The serum cholesterol level and immune response of the animals was checked under, normal, hypercholesterolemic state and after treating the hypercholesterolemic mice with oat extracts. The immune status was checked by employing INOS activity, Phagocytosis, NBT reduction test and ELISA. The oat extracts resulted in immune response enhancement and reduced the cholesterol level in the hypercholesterolemic mice.

Key words: INOS, inducible nitric oxide synthase, NBT, nitroblue tetrazolium chloride, phagocytosis, hypercholesterolemia

INTRODUCTION

High blood cholesterol (hypercholesterolemia) is recognized as a risk factor for coronary heart disease, which is a major health care problem (Zaloga *et al.*, 2006; Mozaffarian *et al.*, 2006). A number of studies have been carried out to screen plants for their medicinal potential and isolate bioactive chemical compounds but only a few studies have been carried out for immunomodulatory potential of plants (Dhillon and Bhatia, 2003). In the 1940's research by Dr. Louis Pillemer yielded a substance that had immune activating properties called Zymosan. Further research by Dr. Nicholas DiLuzio at Tulane University pointed the substance as β -1, 3-D glucan. Glucan, the active component, was isolated from the cell-walls of baker's yeast. Bakers and brewers yeasts, yeast cell walls (bakers yeast glycan) and a protein isolated from yeast (bakers yeast protein) incorporated into the diets partially or totally prevent the elevation of the serum cholesterol (Robbins and Seeley, 1977). Cereals like Barley, Oats have been found to be a good source of dietary fiber including the soluble fiber β -glucan (Onning *et al.*, 1999; Hundemer *et al.*, 1991; Beer *et al.*, 1995; Bell *et al.*, 1999; Yokoyama *et al.*, 1998). Cholesterol lowering effects are reported in studies where barley is the source of beta glucans (McIntosh *et al.*, 1991; Bengtsson and Aman, 1990; Hecker *et al.*, 1998; Kahlon *et al.*, 1993; Kalra and Joad, 2000; German *et al.*, 1996; Oakenfull *et al.*, 1991). The rise in incidence of immunological disorders and hypercholesterolemic patients and the cost and side effect of available drugs raise a need to develop new cost effective therapeutic agents. Keeping in view the increasing incidence of immunological disorders, an inverse relationship between cholesterol level and immune response, an experimental research have been carried out to evaluate the immune response and hypocholesterolemic effect of aqueous oat seeds and leaves extract and commercially available beta-glucan.

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MATERIALS AND METHODS

Animals

Swiss albino mice, 3-4 weeks old weighing 20-25 g were obtained from animal house, Punjab University, Chandigarh. The animals were acclimatized in University animal house conditions for 2-3 weeks before experimentation. Animals used for the experiments were 6-8 weeks old. All the experiments were employed in accordance with Institutional Ethical Committee (IEC, ICMR).

Plant Material

Oat (*Avena sativa*) seeds and leaves were collected and shade dried to prepare extracts.

Aqueous Extract Preparation

Dried and powdered Oat leaves and seeds weighing 5 g were suspended in 50 mL of distilled water and stirred for 5-8 h on magnetic stirrer. Suspension was filtered through What-man filter paper and finally through 0.45 μm Millipore filter and stored at 4°C.

Groups of Animals Used

Animals were divided into following groups:

- Group 1 : Control group (untreated animals)
- Group 2 : Hypercholesterolemic mice (CHO)
- Group 3 : Hypercholesterolemic mice treated with Oat seeds extract (CHO+OSEaq)
- Group 4 : Hypercholesterolemic mice treated with Oat leaves extract (CHO+OLEaq)
- Group 5 : Hypercholesterolemic mice treated with commercially available beta-glucan (CHO+BG)

Inoculation of the Extracts

Mice were inoculated with non-lethal doses of either of extracts i.e., 0.12 g kg^{-1} body weight in 10 doses over a span of 30 days, followed by immunization. Group V mice were inoculated with beta-glucan dose i.e., 20 mg kg^{-1} body weight, followed by immunization.

Induction of Hypercholesterolemia

Hypercholesterolemic condition was induced by injecting single (i.p.) dose of 200 mg kg^{-1} body weight Cholesterol in normal saline.

Immunization

Animals were immunized with 3 doses of BSA intraperitoneally at weekly intervals during the course of extract inoculation.

Follow up of Study

Blood samples of above groups were collected by puncturing retro-orbital plexus and Total Serum Cholesterol levels and High Density Lipoprotein cholesterol (HDL) levels were checked. The blood samples of the animals were collected on 7th day from retro-orbit plexus and centrifuged to separate the serum for humoral immune response. After checking the total serum cholesterol levels, HDL-C and the humoral response of the above groups, the animals were sacrificed on the day 28 after 10 doses of extracts and the effect of immunopotential on hypercholesterolemia was checked.

Estimation of Total Serum Cholesterol

Total serum cholesterol was estimated by the method of Wybenga *et al.* (1970) using commercial kit (Diagnostic Reagent Kit manufactured by Span Diagnostic Ltd., India) The concentration of cholesterol in mg dL^{-1} of the test samples was calculated as:

$$\text{Cholesterol (mg dL}^{-1}\text{)} = \frac{\text{OD of test}}{\text{OD of standard (s)}} \times 200$$

Estimation of High Density Lipoprotein Cholesterol (HDL-C)

HDL-C was determined by one step method of Wybenga *et al.* (1970) using commercial kit, manufactured by Span Diagnostic Ltd., India.

The concentration of serum HDL cholesterol in mg dL⁻¹ of the test samples was calculated as:

$$\text{Serum HDL-C (mg dL}^{-1}\text{)} = (\text{O D of Test (T)/O D of Standard (s)}) \times 50$$

Immunological Parameters

Following tests were carried out to see the immune status of animals:

- Nitroblue Tetrazolium Reduction Test (NBT)
- Inducible nitric oxide synthase Test (INO'S)
- Bactericidal Activity
- ELISA

Nitroblue Tetrazolium Reduction Test (NBT)

The test was carried out by the spectrophotometric method as is given in the hand book of Practical Immunology by Hudson and Hay (1989). The test is based on the principle that NBT on reduction forms blue colored formazen, which is extractable in dioxan. The extracted formazen was measured at 520 nm using dioxan as blank.

Inducible Nitric Oxide Synthase Activity

The test was carried out by employing spectrophotometric method as given by Stuehr and Marletta (1987) is based on the principle that activated immunocytes express high level of nitric oxide synthase which oxidizes Arginine to Citrulline. The nitric oxide formed was measured spectrophotometrically at 540 nm.

Bactericidal Activity

Bactericidal activity of splenocytes was estimated by using the method given in manual of laboratory techniques by Raghuramulu *et al.* (1983). The bactericidal activity of splenocytes was assessed by incubating them in the presence of *E. coli* and the numbers of viable bacteria were measured by taking bacterial suspension as control.

ELISA

Development of the anti Bovine Serum Albumin (BSA) antibodies was observed by ELISA test, as given in the practical manual of Hudson and Hay (1989). The absorbance was measured with ELISA reader (BIORAD) at 492 nm.

Statistical Analysis

Statistical analysis was performed by student's t-test expressed as mean value±SD to assess the change in serum cholesterol level, HDL-C, immune activity of mice from day 0 to day 28.

RESULTS

In the present study the effect of Oat extracts and commercially available Beta-glucan was studied on immune response and cholesterol levels in normal and hypercholesterolemic mice.

Effect of Oat Extracts on Total Serum Cholesterol Levels

The results show that, OSEaq treated and BG treated reduced the serum cholesterol level by 40.1 and 47.59%, respectively while OLEaq resulted in 5.8% decrease in serum cholesterol level as shown in Table 1. Hence the results reveal that OSEaq and BG reduced the total serum cholesterol level of hypercholesterolemic mice.

Effect of Oat Extracts on High Density Lipoprotein (HDL) Cholesterol Levels

The High Density Lipoprotein (HDL) cholesterol levels of hypercholesterolemic mice treated with OSEaq and BG were 32.2 and 30.4% higher than OLEaq as shown in Table 2. The results show that OSEaq and BG treatment resulted in enhancing the HDL cholesterol levels than in OLEaq as compared to the HDL cholesterol levels of hypercholesterolemic mice.

Effect of Oat Extracts on Humoral Immune Response in Hypercholesterolemic Host

The humoral response of hypercholesterolemic animals was checked by using ELISA. The results of ELISA show that antibody titre in hypercholesterolemic mice was 2 times lower than that of control animals. The antibody titre in hypercholesterolemic mice treated OSEaq and BG was 4 times more as compared to control (untreated) and 8 times more as compared to hypercholesterolemic mice (CHO treated). Whereas the antibody titre in hypercholesterolemic mice treated OLEaq was same as compared to control animals and 2 times more as compared to hypercholesterolemic mice (CHO treated) as shown in Table 3.

Effect of Oat Extracts on Cell Mediated Immune Response in Hypercholesterolemic Host

The cell mediated immune response was checked using various immunological parameters like NBT, INOS and Phagocytosis. The results indicate that the values of NBT reduction, INOS and Phagocytosis of hypercholesterolemic mice were lower as compared to control animals as shown in Table 4. However, hypercholesterolemic mice treated with OSEaq and BG extracts showed 1.7 and 2.0 times, respectively an increase in NBT reduction but OLEaq showed 1.1 times increase in NBT

Table 1: Effect of oat extracts and commercially available β -glucan on Total Serum Cholesterol levels in hypercholesterolemic mice

Groups		Total serum cholesterol (mg dL ⁻¹)		
		0 day*	7 day**	28 day***
1	Control	78.80±0.26	78.66±1.35	77.56±1.16
2	CHO	79.69±1.98	289.52±7.27	288.56±7.29
3	CHO+OSEaq	78.68±0.78	288.28±8.51	172.56±2.67
4	CHO+OLEaq	79.17±1.62	289.23±6.82	271.78±8.74
5	CHO+ BG	78.65±0.84	289.74±9.62	151.21±3.98

Values are expressed as mean±SD (n=10), mean differ by p<0.0001, *Total Serum Cholesterol level on day 0 (initial)

** Total Serum Cholesterol level on day 7 (after treatment with Cholesterol), *** Total Serum Cholesterol level on day 28 (after extract treatment of groups III, IV and V)

Table 2: Effect of oat extracts and commercially available β -glucan on HDL Cholesterol levels in hypercholesterolemic mice

Groups		HDL serum cholesterol (mg dL ⁻¹)		
		0 day*	7 day**	28 day***
1	Control	44.38±0.97	43.66±0.24	43.82±0.39
2	CHO	42.15±0.49	34.20±0.74	34.96±0.23
3	CHO+OSEaq	44.98±0.51	50.56±1.74	51.62±0.74
4	CHO+ OLEaq	43.82±0.67	35.76±0.15	35.00±0.74
5	CHO+ BG	44.65±0.94	54.35±0.33	50.25±0.98

Values are expressed as mean±SD (n=10), mean differ by p<0.0001, *HDL-C level on day 0 (initial), **HDL-C level on day 7 (after treatment with Cholesterol), *** HDL-C level on day 28 (after extract treatment of groups III, IV and V)

Table 3: Effect of oat extracts and commercially available β -glucan on Humoral immune response in hypercholesterolemic mice

Groups		Antibody titre
1	Control	1:16
2	CHO	1:8 (2↓)
3	CHO+OSEaq	1:64 (4↑)
4	CHO+OLEaq	1:16 (=)
5	CHO+BG	1:64 (4↑)

The figure in parenthesis reveals the number of times increase (↑), decrease (↓) or equal to control (=).

Table 4: Effect of oat extracts and commercially available β -glucan on Cell-mediated immune response in hypercholesterolemic mice

Groups		NBT reduction (%)	INO'S (%)	Phagocytic activity (%)
1	Control	33.51±0.73	36.74±0.66	25.34±0.23
2	CHO	29.09±0.34	31.95±0.28	20.35±0.38*
3	CHO+OSEaq	58.79±0.57 (1.7↑)	62.95±0.85 (1.7↑)	61.09±0.40(2.3↑)
4	CHO+OLEaq	38.33±0.50 (1.1↑)	39.30±0.37 (1.1↑)	28.56±0.58*(1.1↑)
5	CHO+BG	67.45±0.27 (2.0↑)	69.97±0.27 (2.0↑)	68.30±0.16 (2.6↑)

The figure in parenthesis reveals the number of times increase (↑), decrease (↓) or equal (=) to CHO, Values are expressed as mean±SD (n = 10), mean differ by $p < 0.0001$, *Where $p = 0.0004$

reduction when compared with hypercholesterolemic mice. Similarly, INOS activity was 1.7 and 2.0 times more in hypercholesterolemic treated with OSEaq and BG, respectively, OLEaq showed 1.1 times increase as compared to hypercholesterolemic mice. Higher Phagocytic activity i.e., 2.3 and 2.6 times more was observed in hypercholesterolemic mice treated with OSEaq and BG respectively but OLEaq showed 1.1 times increase in Phagocytic activity as compared to hypercholesterolemic mice (Table 4).

DISCUSSION

The present study was conducted to see the impact of Oat Extracts on hypercholesterolemia and immune response. Hence our results reveal that OSEaq and BG reduced the cholesterol level of hypercholesterolemic mice and enhanced the HDL of hypercholesterolemic mice. Present results corroborate the earlier findings (Brown *et al.*, 1999; Davidson *et al.*, 1991; Kalra and Joad, 2000). Brown *et al.* (1999) studied that various soluble fibers reduce total cholesterol levels but HDL cholesterol were not significantly influenced by soluble fiber. Davidson *et al.* (1991) observed that β -glucan in oat meal and oat bran could lower the total cholesterol levels. Kalra and Joad (2000) observed that barley β -glucan based diet fed to rats caused significant ($p < 0.05$) reductions in the levels of the total cholesterol and significant elevation in the levels of HDL-cholesterol in serum. As shown in another study that twenty-one mildly hypercholesterolemic men were randomly provided with either barley as a source of β -glucan, or wheat (which contains largely cellulose insoluble fibers) for four weeks. Only the barley group showed a significant fall in plasma total cholesterol and in LDL-cholesterol (McIntosh *et al.*, 1991). A study at the University of Massachusetts on 15 obese and hypercholesterolemic men found that supplementation with 15 g of β -glucan fiber derived from yeast daily for eight weeks significantly reduced total cholesterol (by 6%) and LDL-cholesterol (by 8%). (Newman *et al.*, 1989). In present results the test groups show enhancement in immune response. This supports the earlier findings that the β -glucan and oats might modulate and stimulate immune response (Chihara, 1992; Bousquet *et al.*, 1989; Czop, 1986). Chihara (1992) observed that β -glucan appears to represent Host Defence Potentiators (HDPs), which can restore or augment the ability of responsiveness of the host to lympho-cytokines or other intrinsic bioactive factors through maturation, differentiation or proliferation of the important cells for host defence mechanisms. Czop (1986) showed that β -glucan stimulate the antigen-presenting cell function of macrophages therefore, it stimulates the overall immune function. Bousquet *et al.* (1989) studied that β -glucans enhance both non-specific host defense and cellular immune response.

It is concluded that the oat seeds extract reduces the serum cholesterol level and enhances the immune activity and an inverse correlation exists between cholesterol level and immune response. Thus, it is a better alternative to commercially available β -glucan that potentiates immune system.

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