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ORIGINAL ARTICLE

In Vivo Studies On The Immunomodulatory Potential Of Aqueous Oat Extracts

DHILLON P, BHATIA A. ***

ABSTRACT

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 done to find out the immunomodulatory potential of plants. Beta-glucan, one of the chemical constituents of fungi, has been shown to be an immunopotentiating agent. Beta-glucan has been reported to be found in cereals. Hence, in the present study, attempts have been made to evaluate the immunomodulatory potential of oat, a cereal containing beta-glucan. Aqueous extracts of seeds and leaves of oat were evaluated *in-vivo* in swiss albino mice for their immunomodulatory potential. Nitro Blue Tetrazolium reduction (NBT), Inducible Nitric Oxide Synthase (INOS), Phagocytosis (bactericidal activity) and ELISA were various tests employed to assess the effect of oat extracts on the immune status of animals. The results revealed that both the aqueous oat seed extract, as well as its leaf extract were immunopotentiating, the former being more bioactive.

Key Words: Oat, Inducible Nitric Oxide Synthase, Nitro Blue Tetrazolium reduction (NBT), Phagocytosis, ELISA.

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INTRODUCTION

With the increase in the incidence of immunological disorders due to immunosuppressive or inflammatory diseases, there is a need to develop new immunotherapeutics which are cost effective and have a wide range of disease curing potential. 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 to check the immunomodulatory potential of plants [12].

Beta-glucan is one of the chemical constituents of fungi, which shows immunopotentiation. Beta-glucan has been reported in cereals [4], but hardly any work has been carried out to see the immunomodulatory potential of the crude extracts of cereals. Hence, in the present study, attempts have been made to evaluate immunomodulatory potential of aqueous oat extracts.

MATERIALS AND METHODS

Animals: Swiss albino mice, 3-4 weeks old and weighing 20-25 gms were obtained from the animal house, Punjab

University, Chandigarh, and were maintained on a standard lab diet and water. The animals were acclimatized to the 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 the institutional ethical committee.

Plant Material: Oat seeds and leaves were collected and shade dried to prepare extracts.

Aqueous Extract Preparation: Dried and powdered Oat leaves and seeds weighing 5gms were suspended in 50ml of distilled water and stirred for 5-8 hrs on a magnetic stirrer. The suspension was filtered through Whatman filter paper, and finally through 0.45µm Millipore filters, and was stored at 4°C.

Groups of Animals Used:
Animals were divided into the following groups.
Group I: Control group.
Group II: Aqueous Oat seeds extract treated animals (OSEaq)
Group III: Aqueous Oat leaves extract treated animals (OLEaq)

Inoculation of the extracts:
Mice were inoculated with non-lethal doses of either of extracts. Extracts were inoculated in each animal in ten equal doses over a span of 30 days. The volume of each dose was restricted to 0.2ml, so that each mouse received a total dose of 2ml, i.e. 0.12 g/kg body weight in 10 doses, followed by immunization.

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

Follow up of study: After having completed the schedule of extract inoculation, the animals were sacrificed. The spleens of the animals were removed to study the immunological parameters. The spleens were perfused with MEM, and then teased to get splenocytes.

Immunological Parameters:
The 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

[1] **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 [6]. The test is based on the principle that NBT on reduction, forms a blue coloured formazen which is extractable in dioxan. The extracted formazen was measured at 520nm using dioxan as blank.

[2] **Inducible Nitric Oxide Synthase Activity:** The test was carried out by employing a spectro-photometric method as has been put together by Stuehr and Marletta[13], based on the principle that activated immunocytes express high level of nitric oxide synthatase which oxidizes Arginine to Citrulline. The nitric oxide formed, was measured spectrophotometrically at 540nm.

[3] **Bactericidal Activity:** The bactericidal activity of the splenocytes was estimated by using the method given in the manual of laboratory techniques by Raghuramulu et al[8]. The

bactericidal activity of splenocytes was assessed by incubating them in the presence of *E.coli,* and the number of viable bacteria were measured by using the bacterial suspension as control.

[4] **ELISA:** Development of the anti BSA albumin antibodies was observed by an ELISA test, as given in the practical manual of Bhatia A.[3]. The absorbance was measured by using an ELISA reader (BIORAD) at 492 nm.

Statistical analysis: Statistical analysis was performed by student's t-test to access the effect of the oat extracts on immune response.

RESULTS

Effect of Oat extracts on Humoral Immune Response: (Table/Fig. 1)

The antibody titres of the treated extract and control animals were measured by ELISA. The results revealed a 4 times increase in the antibody titre in OSEaq treated animals, but there was no significant increase in the antibody titre in OLEaq treated animals as compared to control.

Table / Fig 1: Effect of Oat extracts on Humoral Immune response.

Group		Antibody Titre
I	Control	1 : 16
II	OSEaq	1 : 64 **(4↑)**
III	OLEaq	1 : 16 **(=)**

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

Effect of Oat extracts on Cell-mediated Immune Response:[Table/Fig.2 and Table/Fig. 3]

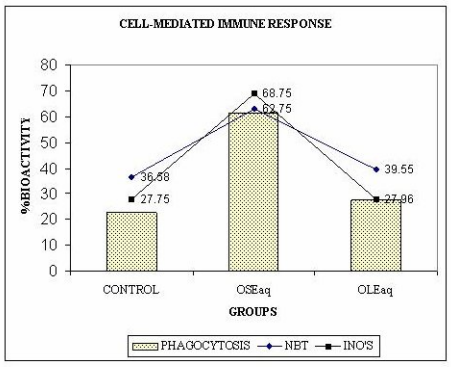
The tests employed to evaluated cell-mediated immune response were NBT reduction test, INO'S activity, and Phagocytic activity. The values of NBT reduction, INO'S activity, and Phagocytic activity were higher in Oat seeds extract treated animals, as compared to the OLEaq treated animals and untreated animals[Table / Fig. 2], [Table/ Fig. 3]. The NBT reduction was 1.7 times more in OSEaq treated mice (62.75%), and was 1 time more in OLEaq treated mice (39.55%), as compared to the control animals (36.58%). Similarly, a 2.4 times increase in INO'S activity was observed in the OSEaq extract treated animals(68.75%), and a 1.0 time increase in OLEaq extract treated animals (27.96%), as compared to control animals (27.75%). The Phagocytic activity was 2.6 times higher in OSEaq treated animals (61.35%), and 1.2 times increase in OSEaq treated animals (27.71%), as compared to untreated mice (22.86%).

Table / Fig 2: Effect of Oat extracts on Cell-mediated Immune response

Group		NBT reduction (%)	INOS (%)	Phagocytic Activity (%)
I	Control	36.58 ± 0.30	27.75 ± 0.70	22.86 ± 0.61
II	OSEaq	62.75 ± 0.42 **(1.7↑)**	68.75 ± 0.70 **(2.4↑)**	61.35 ± 0.90 **(2.6↑)**
III	OLEaq	39.55 ± 0.43 **(1.0↑)**	27.96 ± 1.15 **(1.0↑)**	27.71 ± 0.50 **(1.2↑)**

Values are expressed as mean ± SD (n=10), mean differ by p<0.05

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



Table/Fig 3: EFFECT OF OAT EXTRACTS ON CELL-MEDIATED IMMUNE RESPONSE

DISCUSSION

The present study was conducted to evaluate the immunomodulatory potential of a beta-glucan containing plant, Oat (*Avena sativa*). Aqueous leaves and seeds extracts were evaluated for their immunomodulatory activity in mice, by employing NBT reduction [6], INO'S activity [10], Phagocytosis (Bactericidal activity) [8] and ELISA [3] .Results reveal that both leaves as well as seed extracts enhanced the immune activity of the host. The aqueous extract of oat (*Avena sativa*) seeds show more immunopotentiating activity or modulation of cell-mediated immune response, as compared to the aqueous extract of oat (*Avena sativa*) leaves extract.

The in-vivo immunomodulatory properties of aqueous extracts of oat (*Avena sativa*) seeds and leaves were evaluated. Earlier studies have revealed that beta-glucan from fungi has immunomodulatory activity [13]. On the other hand, beta-glucan has been reported to be present in cereals including oat [5]. The results of our study reveal more bioactivity in seed extract than leaf extract. Literature also shows that seeds have more beta-glucan than leaves. Hence, the better activity of seeds in our study could be mostly due to their beta-glucan content. Many studies of plants have been carried out using crude extracts [1],[9],[11] to check their bioactivities. Even in ayurvedic[12], unani[2] or Chinese [7],[13] medications, traditional formulations containing combinations of various herbs or herbs in combination with minerals have been used. Although it may be rational to use a single plant or its single constituents, it has been a general experience that the total plant extract shows more efficacies as compared to single constituents [9]. Moreover, OSEaq can be employed as such, rather than purified BG, because of its good immunopotentiating activity and cost effectiveness. The present findings are significant for the development of alternative, inexpensive and perhaps safer strategies to boost the immune system.

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