



β -glucan: Immune boosting potential and antioxidant candidate

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ABSTRACT

In literature, β -glucan is abundantly found in oats and currently used as well as tested against various varieties of food products. The objective of our study is to evaluate its immune-boosting potential of β -glucan extracted from oats and tested against specific protein antigen i.e., bovine serum albumin (BSA) and also measure its antioxidant activity. For these studies, β -glucan using variable concentration and tested in human whole blood samples (exposed with an optimized concentration of bovine serum albumin, BSA) and examined its immune-boosting potential. In contrast, estimation of Th1 (IFN-gamma) and Th2 (IL-4) cytokines were also measured from cell culture supernatant and also measured its antioxidant potential through DPPH assay. The results showed that β -glucan enhanced immune-boosting activity in a concentration-dependent manner against BSA. In contrast, there is enhancement in Th2 (IL-4) cytokines in a dose-dependent manner, but there is no significant effect in Th1 (IFN-gamma) as compared to control. In addition, β -glucan showed drastic enhanced antioxidant potential, which is confirmed through DPPH assay. The data demonstrate that β -glucan showing immune-boosting properties and antioxidant potential.



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INTRODUCTION

Dietary fiber is one of the most crucial components of plant material, especially for diet purposes (Charalampopoulos *et al.*, 2002). This component is highly resistant to enzymatic digestion e.g., cellulose, non-cellulosic polysaccharides (includes hemicellulose, pectic substances, gums, mucilages,

and non-carbohydrate component lignin). Most of the diets are highly rich in fiber content e.g., cereals, nuts, fruits, and vegetables, and showed some positive effect with respect to human health, and its consumption rate is directly correlated in order to reduce the burden of several diseases. In short, dietary fiber is mainly seen as well as reported in functional foods (e.g., bakery, drinks, beverages, and meat products) (Charalampopoulos *et al.*, 2002; Demirbas, 2005; Emmons and Peterson, 2001; Esposito *et al.*, 2005).

As per the literature, carbohydrate, especially dietary fiber components, played an important role, especially in the field of vaccine adjuvants, and also claimed them its immunopotentiator properties (Mirza *et al.*, 2017). One of the carbohydrates i.e., glucan, basic structural units and is reported in cell walls of higher plant, along with algae, fungi, yeast, and several other bacterial species. In addition, β -D-glucans are reported as naturally occurring soluble dietary fiber and also used as

a medicine, including cosmetics and food industry (Tada *et al.*, 2008; Vetvicka and Vetvickova, 2007; Talati *et al.*, 2009). In contrast, β -D-glucan is mainly reported as well as isolated from mushrooms and claimed them as one of the non-digestible components of carbohydrates. In short, glucans are considered to be biological response modifiers (immunomodulators have the capacity to stimulate both innate and specific immunity) exerting a variety of immunobiological or immunopharmacological properties (Mirza *et al.*, 2017; Tada *et al.*, 2008; Vetvicka and Vetvickova, 2007; Talati *et al.*, 2009).

According to the physical properties e.g., viscosity related to β -D-glucans, which is mainly dependent on dietary fiber and its solubility along with molecular weight (Esposito *et al.*, 2005; Mirza *et al.*, 2017; Tada *et al.*, 2008; Vetvicka and Vetvickova, 2007; Talati *et al.*, 2009). So, these dietary fibers are mainly linked with a specific type of cancer i.e., colorectal cancer, and these findings are already reported by various researchers (Lipkin *et al.*, 1999). So, various researchers already proposed as well as recommend them to eat high-fiber food contents such as whole grains, vegetables, and fruits in order to reduce the risk of cancer, but do not expressly recommend the use of fiber supplements. In this regard, Food and Drug Administration (FDA) also recommended and allowed them to use this high fiber content in food products and should be able to reduce the risk of heart disease (FDA, 2006).

One of the most important immunobiologically active compounds i.e., β -glucan, called them biological response modifiers (Novak and Vetvicka, 2009). It may play an important role, especially in cancer treatment, infection and its immunity, reduction in stress, restoration of damaged bone marrow, etc. In contrast, β -glucan is one of the most valuable functional types of ingredient which provide better physiological response and showing several health-promoting applications (Novak and Vetvicka, 2009; Bohn and Bemiller, 1995).

Generally, extraction conditions often mainly affect the quality, including quantity along with molecular weight, viscosities, and other physiochemical properties. Therefore, research should be more focus on its applications related to the utilization of β -glucan for the development of new types of products. Similarly, more immunobiological research is required to understand the mechanism by which β -glucan enhances the immune system (Novak and Vetvicka, 2009; Bohn and Bemiller, 1995). In general, the existence of β -glucan in oats is reported, especially in breakfast cereals and snacks. The purpose of these studies in order to investigate the effect of oat

β -glucan and determined its antioxidant potential and also measures its immune-boosting properties against specific protein antigen BSA.

MATERIALS AND METHODS

Selection of samples

Oats were included as one of the most common as well as commercial cereal source of β -glucan. These oats were selected for these studies in order to estimate the β -glucan content. Generally, these oats are available in flakes form in the market. For these studies, we measured β -glucan content from oats sample and measuring its antioxidant activity using DPPH assay and also determining immune-boosting potential against specific protein antigen i.e., BSA.

Extraction of β -glucan from oats

β -glucan was extracted from oats sample using various chemicals (i.e., ethanol, sodium bicarbonate, citric acid). Firstly, an oats sample (200 g) was taken in a flask, and this sample was refluxed with 80 percent ethanol. Then mixing the oats sample with water in the ratio of 1:10 and then keep the sample in a hot stirring plate along with magnetic stirrer for 90 minutes at 55 °C. Thereafter, centrifuge these samples at 15000 rpm for 10 minutes at room temperature. Collect the supernatant and adjust pH 8.5 with sodium bicarbonate and stirred on a hot plate for 30 min at 55 °C. Slightly cool down the solution and adjust the temperature at 37 °C and then centrifuge the sample at 15000 rpm for 10 minutes. Thereafter, the supernatant was collected and adjust the pH at 4 with citric acid. Again, centrifuging (15000 rpm for 20 minutes) and collect the supernatant, finally mixed with ethanol (80 %) in the ratio of 1:1. Incubate the sample for 20 minutes at room temperature. So, centrifuging (12000 rpm, 4°C) these samples and collects the pellet using vacuum drier.

Estimation of Oat β -glucan

Oat β -glucan standard samples were purchased and prepared for the solution for experimentation purposes. Weighing (0.525 g; 95% purity) of the pure β -glucan standard into a 100 ml beaker and reconstituting with ethanol (5 ml) and deionized water (85 ml) by simultaneously stirring and heating the mixture on a hot plate at 120 °C. Finally, β -glucan was completely dissolved, and the solution was allowed to cool to room temperature. Then the solution was adjusted to 100 ml with deionized water. The solution had a β -glucan concentration of 500 mg/l (i.e. 0.5 mg/ml) and was manually diluted to a final concentration of 0.25 mg/ml, 0.125 mg/ml and 0.0625 mg/ml. For these studies, alcohol precipitation of β -glucan was more preferable because it

only provides the highest yield. In addition, enzymatic extraction provides the highest yield as well and also responsible for removing starch, fat, and minerals without significantly affecting its physicochemical properties. Prepare the standard curve and calculate its β -glucan extraction from oats sample will be measured in mg/ml. B -glucans have β -D-glucose polysaccharides, which are naturally occurring primarily in cereals and bacteria (Figure 1). Its physicochemical properties are altered based on its source and also depending on characteristics of their primary structure, including linkage-type, degree of branching, molecular weight, and conformation. Its molecular weight of extracted samples i.e., β -glucan were analyzed through MALDI (Matrix-assisted laser adsorption or desorption ionization technique).

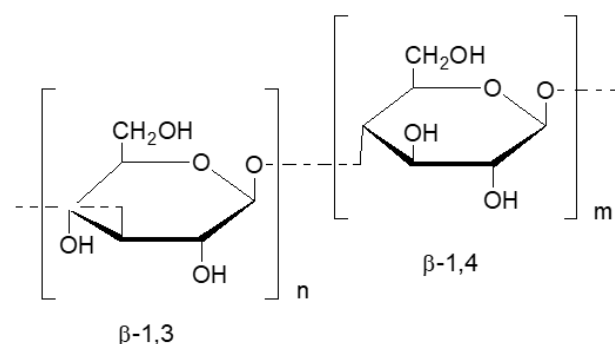


Figure 1: Structure of β -glucan

Immune boosting potential activity

For determining its immune-boosting potential using a variable concentration of β -glucan against an optimized dose of BSA in lysed human whole blood. For these studies, lysed human whole blood (10^6 cells/ml; $100 \mu\text{l}$) were taken in 96 well flat bottom tissue culture plate and then exposed to BSA (2 mg/ml; $50 \mu\text{l}$) along with variable concentration of β -glucan (0.1- 100 mg/ml, $50 \mu\text{l}$) for determining its immune-boosting properties.

These cells containing β -glucan and BSA were grown properly in nutritive rich media with pH 7.4 and maintained at 37°C for 24 h in incubator. After incubation (37°C , 24 h), media in the wells was collected for the estimation of Th1/Th2 cytokines (using BD optic Kit) (Maheshwari et al., 2017; Gupta et al., 2006) and then add fresh medium containing MTT solution (2.5 mg/ml; $10 \mu\text{l}$) and incubate the plates for another 4h at 37°C .

Thereafter, media-containing MTT was removed after centrifuging, and formazan crystals were appeared at the bottom and dissolved in dimethyl sulphoxide (DMSO). The optical density (OD) was recorded in a microplate reader at 570 nm (Mahesh-

wari et al., 2017; Gupta et al., 2006).

An antioxidant activity using DPPH free radical assay

In an effort to determine its antioxidant effect in β -glucan, which is extracted from oats sample, and it is determined through DPPH free radical assay (Kha-juria et al., 2007). In this study, β -glucan was reacted with the stable DPPH radical in an ethanol solution. So, the reaction mixture consisted of β -glucan (0.5 ml), absolute ethanol (3 ml) and DPPH radical solution (0.3 ml dissolved in 0.5 mM ethanol). For these studies, DPPH reacts with an antioxidant compound, which can donate hydrogen; it is reduced. The changes in color (from deep violet to light yellow) were read [Absorbance (Abs)] at 517 nm after 100 min of reaction using a UV-VIS spectrophotometer. For these studies, ethanol (3.3 ml) and sample (0.5 ml) selected as blank, whereas the control solution contained ethanol (3.5 ml) and DPPH radical (0.3 ml) solution. The scavenging activity percentage (AA %) was determined by using this equation i.e.

Antioxidant activity =

$$100 - \left[\frac{(\text{Sample absorbance} - \text{Blank absorbance}) \times 100}{\text{absorbance control}} \right]$$

Statistical analysis

The difference between control and variable concentration of β -glucan is determined through Bonferroni multiple comparison test (One way ANOVA).

RESULTS AND DISCUSSION

Estimation of B -glucan

B -glucan, a polysaccharide that is composed of glucose units linked together by beta glycosidic linkage having (1,3) and (1,4) bonds. The most documented nutritional benefit of β -glucan is flattening postprandial glucose, insulin, and cholesterol rise. FDA has acknowledged and recommended 3gm of β -glucan per day for achieving the health benefits. As β -glucan has shown to have a positive influence on human health, in the present study, it was estimated in oats. The estimated amount of β -glucan from the oats sample was found to be 61.87 mg/ml.

Immune boosting potential

The effect of the variable concentration of β -glucan on lysed human whole blood containing exposure of optimized concentration of BSA was shown in Figure 2. The results showed that β -glucan claimed its immune-boosting potential in the form of lymphocyte proliferation at a higher concentration as compared to control. BSA used as a standard for

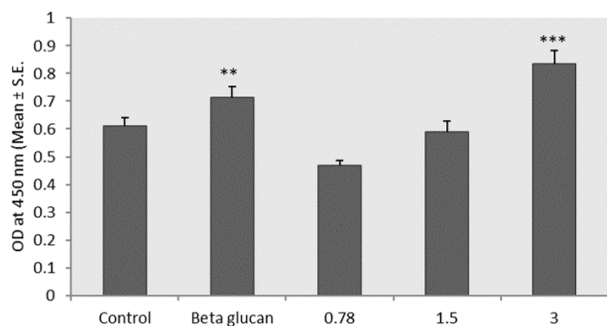


Figure 2: Lymphocyte proliferation assay. To determine the effect of variable doses of β -glucan (0.78 – 3 mg/ml, 50 μ l) along with an optimized dose of BSA in lysed human whole blood. Values are expressed as Mean \pm S.E. The difference between the controls versus variable doses of β -glucan is determined by one-way ANOVA test (Bonferroni multiple comparison tests) *P<0.05; **P<0.01, *P<0.001**

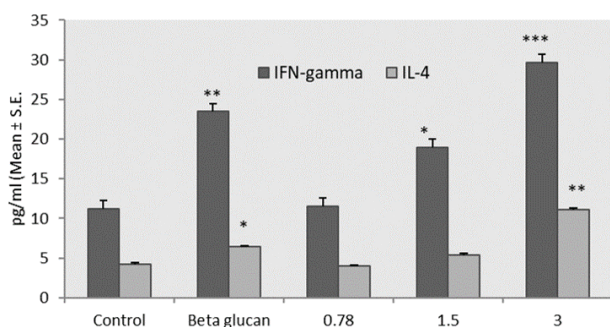


Figure 3: ELISA assay. To determine the level of Th1 and Th2 cytokines from cell culture supernatant using BD optic Elisa Kit. In order to determine the effect of variable doses of β -glucan (0.78 – 3 mg/ml, 50 μ l) along with an optimized dose of BSA in cell culture supernatant from lysed human whole blood. Values are expressed as Mean \pm S.E. The difference between the controls versus variable doses of β -glucan is determined by a one-way ANOVA test (Bonferroni multiple comparison tests). *P<0.05; **P<0.01, *P<0.001**

these studies, and there is a moderate enhancement in proliferation rate as compared to control. Overall, these studies claimed that β -glucan showed immune-boosting potential against BSA. For further confirmation of these studies, the effect of β -glucan from lysed human whole blood cell culture supernatant for estimation of Th1 (IFN-gamma) and Th2 (IL-4) cytokines as shown in Figure 3. The results showed that β -glucan claimed its enhancement in Th1 and Th2 profile at a higher concentration as compared to control.

Antioxidant potential

DPPH method is mainly applied to pertain to determine the ability of β -glucan to act as free radical scavengers or hydrogen donors and to evaluate or determining its antioxidant capacity. In this regard, the IC50 parameter is applied and used for results interpretation from the DPPH method. The results of these studies, as shown in Figure 4 and showed the DPPH radical scavenging activity of β -glucan. In other words, DPPH considered them as stable free radical, which is mainly reduced in the presence of hydrogen donating antioxidants. So, its scavenging ability of β -glucan for free radicals of 1, 1-diphenyl 1-2-picrylhydrazyl (DPPH) showed remarkable scavenging activities. The highest scavenging activity was found at a higher concentration of β -glucan as compared to control. Overall, these studies claimed that β -glucan showed higher antioxidant activity.

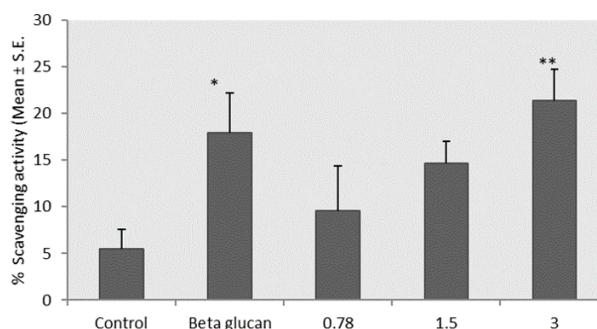


Figure 4: Antioxidant potential using DPPH assay. Values are expressed as Mean \pm S.E. The difference between the controls versus variable doses of β -glucan is determined by one-way ANOVA test (Bonferroni multiple comparison tests) *P< 0.05; **P<0.01, *P<0.001**

β -glucan is one of the most important components which is reported in the cell wall of pathogenic bacteria (e.g., *Aspergillus fumigatus*) and fungi (*Saccharomyces cerevisiae*). In literature, β -glucan is considered them as non-digestible carbohydrates and also identified them as biological response modifiers with anti-tumor properties (Charalampopoulos *et al.*, 2002; Demirbas, 2005; Emmons and Peterson, 2001; Esposito *et al.*, 2005). In general, medical practitioners also recommended β -glucan (injected subcutaneously; shot under the skin) for treating/reducing the size of skin tumors resulting from cancer and also used as a food additive, especially salad, frozen desserts and cheese spreads. These studies should be further extended pertaining to translate β -glucan research to the clinical level. In the present study, our main objective is to analyze the effect of β -glucan isolated from oats and determined its immune-boosting properties against specific protein antigen, BSA, and antioxidant activity

through DPPH assay.

We determined the effect of BSA plus β -glucan was associated with an enhancement in proliferative response as compared to BSA control. The proliferative response of β -glucan associated with BSA was dose-dependent. When the variable concentration of β -glucan was used, the responses against BSA were enhanced. Understanding the mechanisms of β -glucan might lead to the development of immune-boosting agents and also used for various therapies or disorders, which is mainly caused by genetic and environmental factors. In addition, these studies suggested that β -glucan induces both Th1 and Th2 immune responses, because IFN-gamma production is dependent on Th1 cells, whereas IL-4 on Th2 cells (Maheshwari *et al.*, 2017; Gupta *et al.*, 2006). So, these studies are also supported by this finding and considered that secretion of Th1 cytokines (IFN- γ and IL-2) and Th2 cytokines (IL-4 and IL-5) was augmented by β -glucan. In other words, β -glucan showed an immune-boosting effect on Th1 and Th2 immune responses. Overall, β -glucan moieties binding with a specific type of receptors, especially on monocyte-derived macrophages, which triggers their activation and enhances the mediators of proinflammatory cytokines that stimulate Th1 (IFN-gamma) immune responses. In addition, it also enhanced IL-4 production as compared to control. Further immunological studies are required pertaining to evaluate the effect of β -glucan against specific protein antigen hepatitis and typhoid and determining its proliferative response along with an estimation of Th1 and Th2 cytokines.

One of the assays i.e., DPPH free radical scavenging method, is mainly used for evaluating its antioxidant activity or potential of particular compound isolated from oats. The main advantage of this method i.e., DPPH, is mainly applied in order to examine both hydrophilic and lipophilic antioxidants (Khanjuria *et al.*, 2007; Gupta *et al.*, 2016). In spite of the higher antioxidant activity is reported in various medicinal plant products, but its shelf life is so short and is mainly affected through various storage conditions (temperature, time, light exposure). This is one of the simplest methods, where a particular compound or pure molecule is mixed with DPPH solution, and absorbance is recorded after a defined period. In view of this, we determined the activity of β -glucan from oats, and results claimed its existence of antioxidant activity. This activity is mainly determined through DPPH and also dependent on the source and type of extraction method used. Overall, these studies claimed that β -glucan was significantly higher with antioxidant activity along with other benefits, which may contribute to enhancing

its health and beauty.

CONCLUSIONS

Based on these studies and results presented here, this is concluded that β -glucan exhibit immune-boosting potential in the form of proliferation rate against specific protein antigen. Further confirmation through estimation of Th1 and Th2 cytokines along with the considerable amount of antioxidant radical scavenging activity (DPPH assay) and possess substantial amounts of phenolic compounds. In short, β -glucan should be considered as one of the good sources of antioxidants and immune-boosting potential against various infectious agents.

Authors Contribution

This work was carried out in the collaboration between five authors. Dr. Amit Gupta designed the study, wrote the protocol and interpreted the data where Abhishek (Student of B.Sc. Food Tech Department, 5th Semester), Aanchal and Tanya (M.Sc Microbiology, 3rd Semester) anchored the field study, gathered the initial data related to his/her project work under Dr. Amit Gupta guidance and performed preliminary data analysis. Dr. Amit Gupta and Dr Ajam Shaikh managed the literature searches whereas Dr. Amit Gupta produced the initial draft. The final manuscript has been read and approved by all authors.

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