

Extraction of β -glucan of *Hericum erinaceus*, *Avena sativa* L., and *Saccharomyces cerevisiae* and *in vivo* evaluation of their immunomodulatory effects

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Abstract

Beta-glucan (BG) is one of the most common types of polysaccharides, and is a potent immune activator. It is present in microbes, plants, and mushrooms. The immunomodulatory properties of BG are varied among different species. This paper explains the composition of BG from yeast (*Saccharomyces cerevisiae* HII31), oat (*Avena sativa* L.) and mushroom (*Hericum erinaceus*), and also describes *in vivo* immunomodulation and changes in the antioxidant capacity of BG. *In vivo* studies were conducted in BALB/cMlac mice, and changes in cytokines level, antioxidant enzymes, and total antioxidant capacity were evaluated. About 91.42 ± 5.07 , 86.90 ± 3.44 , and $84.43 \pm 4.10\%$ of BG were recorded in yeast, mushroom, and oat samples, respectively, with 4-5% of α -glucan in all the samples. The band area range ratio analysis indicate that yeast and mushroom have a high content of β -1,3-glucan followed by β -1,6-glucan, whereas oat recorded a high content of β -1,4-glucan. Yeast-BG stimulated the expression of IL-6, IL-17, IFN- γ , IL-10, and TGF- β effectively. Moreover, antioxidant capacity was enhanced during yeast-BG supplementation in a dose-dependent manner than oat and mushroom BG. This study reveals that yeast-BG is a potent immune activator and enhancer of the host's antioxidant capacity than oat, and mushroom-BG.

Keywords: β -glucan; *Saccharomyces cerevisiae* HII31; *Avena sativa* L.; *Hericum erinaceus*; cytokines; antioxidants.

Practical Application: Selection of potent β -glucan with immune enhancing property for the pharmacological applications.

1 Introduction

The word 'glucan' refers to polymers of glucose that include cellulose (β -1,4-glucan). They are branched or unbranched, short or long, soluble or particulate, and in α or β isomeric forms. Beta-glucan (BG) is one of the most common types of polysaccharides (Goodridge et al., 2009; Chan et al., 2009). Fungal BG, in particular the BG of *Saccharomyces cerevisiae*, have variable numbers of 1, 6 branches, and the forms in which the refined glucan molecules exist range from glucose dimers to large insoluble particles. BG from yeast (*S. cerevisiae*), mushroom (*Sclerotium glaucum* and others), bacteria (curdlan from *Alcaligenes faecalis*), and seaweed (laminarin from *Laminaria digitata*) have been reported for their pro- or anti-inflammatory effects (Goodridge et al., 2009).

The host's immune receptors will recognize the carbohydrate moiety of BG and elucidate the defensive response (Brown & Gordon, 2005). The immunomodulatory and cancerostatic properties of BG are established both *in vitro* and *in vivo* against breast, lung, colorectal (Dongowski et al., 2002), and gastrointestinal cancers. BG also has antioxidant properties, and carry out wound healing activities (Petravic-Tominac et al., 2010). Further, it prevents coronary heart disease (Wang et al., 2002), decreases blood glucose levels in diabetic patients, ameliorates

insulin resistance (Hallfrisch et al., 2003; Hlebowicz et al., 2008), controls serum cholesterol levels (Smith et al., 2008), and influences the gut microflora (Tungland, 2003).

Other than the medical field, BG is involved in many other areas, like the food and cosmetic industries. BG can be used in the making of sauces, soups, and beverages. Moreover, it may act as a thickening agent, stabilizer, and emulsifier (Burkus & Temelli, 2000). BG serves as moisture retention agents in cosmetics, especially in creams and lotions for sensitive and irritated skin (Wheatcroft et al., 2002). BG accounts for a significant part of yeast biomass, and is necessary in the performance of cell functions (Bacic et al., 2009). Yeast-BG is also used as an adjuvant with anti-infective or antineoplastic agents, after radiotherapy (Petravic-Tominac et al., 2010). An *in vitro* study revealed that the BG of *S. cerevisiae* can promote TNF- α production and trigger the mononuclear cells, and neutrophils (Olson et al., 1996). It is proven that the consumption of whole grain, low glycemic index, and high dietary fiber diets reduce the development of type 2 diabetes and heart diseases. Thus, oats and barley are the primary choice of cereals enriched with BG. The serum cholesterol-lowering property of the oat and

Received 23 May, 2017

Accepted 26 Feb., 2018

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barley is related to their molecular weight, and the solubility of the BG (Wood, 2007).

Mushrooms are well-known immune-modulators because of the richness of their polysaccharide fractions, primarily BG. Fungal BG can activate the leukocytes, and edible mushrooms have some effects on enterocytes (Volman et al., 2010). *Hericium erinaceus* is better identified for its antimicrobial, antioxidant, antihypertensive, antidiabetic nature and enrichment of protein (Woraharn et al., 2015). *H. erinaceus* is commonly used for the production of fermented mushroom juices, and Lactic Acid Bacteria mediated fermented *H. erinaceus* juices are enriched with L-glutamic acid and γ -aminobutyric acid (Woraharn et al., 2016). Even though the bioactivity of the mushroom depends on the configuration, and content of the BG, information about the BG of *H. erinaceus* is very limited. The structure, quality, and functionally of extracted BG is majorly influenced by the extraction method (Ahmad et al., 2012). The changes in the total antioxidant capacity and antioxidant enzymes level during BG exposure to the host system are not yet illustrated clearly, even though the antioxidant potential of BG has been reported (Kofuji et al., 2012). Thus, the current work deals with the extraction of BG from yeast (*Saccharomyces cerevisiae* HII31), oat (*Avena sativa* L.), and mushroom (*Hericium erinaceus*) and *in vivo* analysis of their immunomodulatory and antioxidant properties. Moreover, changes in the antioxidant capacity of mice during BG supplementation were also studied.

2 Materials and methods

2.1 Materials

Saccharomyces cerevisiae HII31, oat (*Avena sativa* L.), and mushroom (*Hericium erinaceus*) were obtained from Health Innovation Institute, Chiang Mai, Thailand.

2.2 Extraction

The source materials (yeast, oat, and mushroom) were suspended in 5 fold of 1.0 M NaOH and incubated at 80 °C. After shaking for 2 h, centrifugation was carried out at 6,000 \times g, for 25 min at 4 °C. The pellets were washed with distilled water 3 times by centrifugation at 6,000 \times g, for 25 min at 4 °C for cleaning purpose. The obtained pellets were suspended in 5 fold of 1.0 M acetic acid and incubated at 80 °C for 2 h with constant stirring by a stirrer force. After incubation, the suspensions were centrifuged at 6,000 \times g, for 25 min at 4 °C. The process was repeated three times. The collected pellets were dried in absolute ethanol and hot air oven at 60 °C. The dried glucan pellets were stored at 4 °C until analysis (Pengkumsri et al., 2017a).

2.3 Glucan determination and FT-IR

The glucan content of the sample was determined by glucan assay kit as per the manufacturer's instructions (yeast & mushroom, Megazyme, Ireland). The component and ratio of glucan in the extracts were analyzed by Fourier Transform Infrared Spectroscopy (FT-IR). The Spectra (from 400 to 4,000 cm^{-1} at 4 cm^{-1} resolution) were recorded for glucan extracts with FT-IR spectrometer (Nicolet Nexus 470 FT-IR instrument, USA); an average of 32 scans was

computed for each sample. All measurements were acquired in duplicate normalization. The spectra were pre-processed (baseline correction, normalization, and second derivative) with OMNIC 32 software. The Origin Pro 8.0 software was used to calculate the area with curve-fitting method (Pengkumsri et al., 2017a, b).

2.4 Animal procedure

The six-weeks-old male BALB/cMlac mice (n = 60) were randomly divided into ten groups as follows: control (G1; n = 6) received normal diet and water, yeast glucan extract group (100, 150, and 200 mg/kg body weight; G2, G3, G4 respectively; n = 6 each), oat glucan extract group (100, 150, and 200 mg/kg body weight; G5, G6, G7 respectively; n = 6 each), mushroom glucan extract group (100, 150, and 200 mg/kg body weight; G8, G9, G10 respectively; n = 6 each). All mice were exposed (by oral gavage) to the respective glucans and the respective concentrations for seven days, and blood samples were collected after they were sacrificed on the 7th day to analyze the levels of selected serum cytokines (IL-6, IL-10, IL-17, IFN- γ , and TGF- β). Moreover, the liver tissue was collected for antioxidant capacity (TEAC), antioxidant enzymes (CAT, SOD, GPx), and oxidation of lipid (MDA) assessments. Ethical approval was obtained for the animal experiments from the Ethical Committee of Use and Care of Animals, Faculty of Pharmacy, Chiang Mai University (Ref. No. 01/2015 dated 15.09.2015).

2.5 Evaluation of cytokines in the serum samples

The levels of the selected cytokines (IL-6, IL-10, IL-17, IFN- γ , and TGF- β) in the serum samples collected from each experimental group were determined by ELISA as per the manufacturer's instructions (R&D system, USA).

2.6 Preparation of liver extract and determination of protein

The extracted mice livers were prepared as detailed by Guo et al. (2013). Briefly, 1.0 g of a freshly-collected mouse liver was ground in cool condition with 10 mL of Phosphate-buffered saline (PBS; pH 7.0). The liver suspension was centrifuged at 3,500 rpm for 30 min at 4 °C. Then, the supernatant was collected and used for antioxidant capacity, antioxidant enzymes, and oxidation of lipid determinations. Afterward, the protein contents of the samples were determined by Lowry's method with bovine serum albumin (BSA) (20 - 200 $\mu\text{g/mL}$) as a substrate for standard curve preparation (Lowry et al., 1951).

2.7 Evaluation of lipid oxidation, antioxidant capacity, and antioxidant enzymes

The lipid oxidation was measured by determining the concentration of malondialdehyde (MDA) as detailed in a previous report (Pengkumsri et al., 2015). The MDA product was measured using a Multimode Detector (Beckman coulter, DTX 880, USA) at 540 nm. The ng/mg protein of the liver extract was reported.

The 2, 2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assay was performed to determine the total antioxidant capacity (TAC). The results are stated as ng trolox equivalents

antioxidant capacity (TEAC) of mg protein of liver extract. All samples were tested in triplicate (Pengkumsri et al., 2015).

The catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities were measured in the liver extract as reported by Pengkumsri et al. (2015). All experiments were carried out in triplicates, and the values were represented as U/mg protein.

2.8 Statistical analysis

Analysis of variance (ANOVA) was performed, with the confident interval at 95% ($p < 0.05$). The statistical analysis was performed in SPSS 17.0 to check the significant differences in the glucan content of the extracts and the cytokine levels in the serum samples. The Least Significant Difference (LSD) post hoc test was performed to analyze the significant differences in antioxidant activities and $p < 0.05$ was considered as significant.

3 Results and discussion

The yeast, mushroom, and oat samples were subjected to total glucan extraction. About 68 ± 3 , 25 ± 2 , and $26 \pm 2\%$ of yield were recorded in the yeast, mushroom, and oat samples, respectively. The total glucan content of the extracts were found as 97 ± 4 , 92 ± 4 , and $89 \pm 4\%$ in the yeast, mushroom, and oat samples, respectively. About 91 ± 5 , 87 ± 3 , and $84 \pm 4\%$ of β -glucan (BG) were recorded in the yeast, mushroom, and oat samples, respectively, with 4-5% of α -glucan (AG) in all the samples (Table 1). The results suggest that yeast cells have more BG than other tested samples with respect to total yield and percentage of BG content (Table 1).

Isolated glucan samples were subjected to FT-IR analysis with absorption range of $400\text{--}4000\text{ cm}^{-1}$. The specific absorption range for the polysaccharide region, $925\text{--}1175\text{ cm}^{-1}$, was further analyzed by the curve-fitting method to explore the hidden information of the tested samples by Origin Pro 8.0 software. The band areas of yeast, mushroom, and oat glucans are shown in Table 2 with the respective wavelengths, and the bands were assigned as β -1,6-glucans (~ 985 , $\sim 991\text{ cm}^{-1}$), β -1,4-glucans (~ 1038 , $\sim 1041\text{ cm}^{-1}$) and β -1,3-glucan (~ 1111 , ~ 1109 , ~ 1115 , ~ 1159 , $\sim 1161\text{ cm}^{-1}$). The band area range ratio (BARR) of β -1,3-glucan: β -1,4-glucan: β -1,6-glucan in the yeast, mushroom, and oat samples were 2.3:1.0:2.0, 1.9:1.0:1.4, and 1.0:10.5:4.3, respectively (Table 3). The FT-IR (Table 2, 3), and BARR data suggest that yeast and mushroom contain a high content of β -1, 3-glucan followed by β -1, 6-glucan, whereas oat recorded a high content of β -1, 4-glucan. The second derivative of the mean FT-IR spectra, and the normalized FT-IR average spectra of yeast, mushroom, and oat β -glucans are shown in Appendix A (Supplementary data; Figure S1, Figure S2, and Figure S3, respectively).

The composition of glucans from the mushroom *Entoloma lividoalbum* was found to be β -d-glucopyranosyl, 1-3, 1-6 β -d-glucopyranosyl, and 1-3, 6 β -d-glucopyranosyl in a ratio of 1:3:2:1 (Maity et al., 2014), whereas, the water soluble branched β -d-glucan from edible mushroom *Russula albonigra* (Krombh.) Fr. were reported to be in a ratio of 1:2:2:1 (β -d-glucopyranosyl, 1-3, 1-6 β -d-glucopyranosyl, and 1-3, 6 β -d-glucopyranosyl) (Nandi et al., 2014). The present study

also proves that the mushroom *H. erinaceus* contains a high level of 1-3 β -d-glucan (Table 2, 3).

The immunomodulatory properties of the extracted BG samples were assessed. The levels of inflammatory and anti-inflammatory cytokines were assessed. The levels of IL-6 were 685 ± 62 , 659 ± 50 , and $682 \pm 39\text{ pg/mL}$ for the 200 mg/kg yeast-BG, oat-BG, and mushroom-BG interventions, respectively. The levels of IL-17 were 155 ± 15 , 145 ± 15 , and $151 \pm 16\text{ pg/mL}$ for the yeast-BG, oat-BG, and mushroom-BG interventions, respectively. The IFN- γ levels were 238 ± 26 , 203 ± 16 , and $214 \pm 15\text{ pg/mL}$ in mice serum for the yeast-BG, oat-BG, and mushroom-BG intervention-, respectively (Table 4). The data indicate that the yeast-BG stimulated the expression of IL-6, IL-17, and IFN- γ more effectively than oat and mushroom-BG. Interestingly, the oat-BG mediated induction was not enhanced effectively with increased concentration, even though statistically different from the other group.

The anti-inflammatory cytokines (IL-10, and TGF- β) in the mice serum was elevated upon BG interventions. Obviously, yeast-BG induces the maximum level of IL-10 and TGF- β in mice compared to other tested BG samples. The level of IL-10 and TGF- β were 276 ± 18 , and $651 \pm 28\text{ pg/mL}$, respectively in yeast-BG (200 mg/kg) intervention group, whereas the control

Table 1. The yield of glucan extracted from selected sources.

Sources	% of yield	Total-glucan (%, w/w)	α -glucan (%, w/w)	β -glucan (%, w/w)
Yeast	68 ± 3	97 ± 4	5.3 ± 0.2	91 ± 5
Mushroom	25 ± 2	92 ± 4	5.2 ± 0.2	87 ± 3
Oat	26 ± 2	89 ± 4	4.4 ± 0.2	84 ± 4

Table 2. The band area and identified bands by curve-fitting analysis of the FT-IR spectra of yeast, mushroom and oat β -glucan extracts with spectral range of $925\text{--}1175\text{ cm}^{-1}$.

Yeast		Mushroom		Oat		Assigned as
Wave number (cm^{-1})	Band area (%)	Wave number (cm^{-1})	Band area (%)	Wave number (cm^{-1})	Band area (%)	
~ 985	32.24	~ 991	24.59	~ 991	15.53	β -1,6 glucan
~ 1038	15.91	~ 1041	17.96	~ 1038	38.25	β -1,4 glucan
~ 1111	13.26	~ 1109	16.04	~ 1115	0.50	β -1,3 glucan
~ 1161	22.89	~ 1159	17.52	~ 1161	3.14	β -1,3 glucan

Table 3. The band area and ratio of different forms of β -glucan extracts.

Sources	Band area (%)			Range ratio (1:2:3)
	β -1,3-glucan (1)	β -1,4-glucan (2)	β -1,6-glucan (3)	
Yeast	36.15	15.91	32.24	2.3:1.0:2.0
Mushroom	33.56	17.96	24.59	1.9:1.0:1.4
Oat	3.64	38.25	15.53	1.0:10.5:4.3

group displayed 244 ± 21 pg/mL, and 577 ± 15 pg/mL of IL-10, and TGF- β , respectively (Table 4).

The lipopolysaccharide (LPS) mediated enteritis induced in rat, supplemented with high molecular oat-BG (2,179,700 g/mol) showed an improvement in stress oxidative parameters in the spleen, and reduced the lipid superoxides, 7-ketocholesterol level and glutathione disulfide activity (Blaszczyk et al., 2015). The LPS induced enteritis in rat treated with partially purified (75%) low MW oat-BG showed a reduction in the T and B lymphocytes, granulocytes and Tc lymphocytes.

The high MW Oat-BG (2,180,000 g/mol) intervention effectively reduced the lipid peroxidation in naïve control rats, whereas the low MW BG (70,000 g/mol) did the same in the enteritis groups, which suggest that low MW BG supplementation enhanced the antioxidant capacity of the host and facilitates disease recovery (Suchecka et al., 2015). The studies proves that yeast-BG can stimulate the level of TNF- α , IL-6, IL-10, and TGF- β (Olson et al., 1996; Tzianabos, 2000; Engstad et al., 2002). The involvement of BG in immune regulation of colitis condition has been reported (Roda et al., 2011). The water extract of the fruiting body of Oyster mushroom (*Pleurotus ostreatus*) can induce the production of reactive oxygen species and modulate the defense system (Shamtsyan et al., 2004). NF- κ B is a transcription factor which is involved in immune response and regulation. *Agaricus bisporus* polysaccharide, possibly BG, affects and lowers the NF- κ B transactivation in Caco-2 cells (Volman et al., 2010). The structure, composition and the biological properties of oat-BG have been reviewed (Daou & Zhang, 2012). The changes in the immune activation property were due to the compositional differences among the samples. The glucan compounds are either homo or hetero glucans with β (1, 3), β (1,4) and β (1,6) glycosidic bond that plays a vital role in some bioactivity of mushrooms. In cereals (oat and barley), BG is made-up of mixed-linkage (1,3), (1,4)- β -d-glucose units, whereas it is composed of mixed-linkage of (1,3), (1,6)- β -d-glucose units in yeasts and mushrooms. The FT-IR and band area analysis also reveal that the oat-BG is composed more of

β -1,4 glucans, and β -1,6 glucans, whereas yeast and mushroom BG are mainly composed of β -1, 3 glucan, and β -1, 6 glucans (Table 1, 2, 3).

The antioxidant capacity of BG supplemented mice was determined. All the BG-treated mice showed an increase in the trolox equivalent of antioxidant capacity (TEAC), in a dose-dependent manner. The maximum TEAC in yeast BG-200 mg group (4.0 ± 0.3 ng/mg of protein) and a slight increase in TEAC in oat-BG-100 mg group (2.7 ± 0.1 ng/mg of protein) were observed while the TEAC of naïve control was 2.5 ± 0.1 ng/mg of protein. The mushroom glucan displayed a moderate level of enhancement of TEAC in experimental mice (Figure 1A). The ability of the studied BG extracts to inhibit lipid peroxidation was assessed. Like TEAC, yeast-BG groups, oat-BG groups, and mushroom-BG groups showed maximum, minimum, and a moderate level of suppression of lipid peroxidation regarding MDA formation (Figure 1B).

The changes in the major antioxidant enzymes such as catalase, SOD, and GPx during BG supplementations were studied. The levels of all the studied enzymes were increased upon yeast-and mushroom-BG interventions for all the concentrations (100 - 200 mg), whereas oat BG required slightly high concentration (150 - 200 mg) to enhance the enzymes level in mice compared to the naïve control. The maximum level of catalase, SOD, and GPx during yeast BG-200 mg intervention in mice were recorded as 28 ± 2 , 113 ± 9 , and 7 ± 1 U/mg of protein, respectively (Figure 2).

Studies have shown the enhanced bioactivity of BG isolated from other sources like barley, and mushroom. Barley-BG showed a stronger antioxidant activity than the BG of black yeast and oats. The extraction method, and complexity (oligomers, macromolecules) of BG influence the free radical scavenging activity (Kofuji et al., 2012). The glucan of *Gaeastrum saccatum* (mushroom) protects cells from lipid peroxidation, and oxidative (hydroxyl and superoxide) stresses (Guerra Dore et al., 2007).

Table 4. Changes in the selected cytokines (IL-6, IL-10, IL-17, INF- γ , and TGF- β) level in mice serum after glucan treatment.

Group	IL-6 (pg/mL)	IL-10 (pg/mL)	IL-17 (pg/mL)	INF- γ (pg/mL)	TGF- β (pg/mL)
Control	582 ± 59	244 ± 21	137 ± 12	134 ± 22	577 ± 15
YG-100	$660 \pm 60^{***}$	261 ± 24^d	141 ± 12^e	$209 \pm 25^{d***}$	591 ± 24^e
YG-150	$670 \pm 31^{b***}$	$270 \pm 28^{b***}$	148 ± 14^e	$220 \pm 20^{b***}$	$631 \pm 31^{b***}$
YG-200	$685 \pm 62^{a***}$	$276 \pm 18^{a***}$	$155 \pm 15^{a***}$	$238 \pm 26^{a***}$	$651 \pm 28^{a***}$
OG-100	$643 \pm 54^{d***}$	247 ± 20^e	140 ± 15^f	$165 \pm 12^{b***}$	581 ± 38^e
OG-150	$653 \pm 52^{c***}$	250 ± 25^f	142 ± 14^e	$186 \pm 20^{c***}$	585 ± 29^e
OG-200	$659 \pm 50^{c***}$	259 ± 22^e	145 ± 15^d	$203 \pm 16^{d***}$	$601 \pm 24^{d*}$
MG-100	$660 \pm 42^{c***}$	252 ± 20^f	141 ± 15^e	$173 \pm 9^{b***}$	586 ± 21^e
MG-150	$671 \pm 34^{b***}$	261 ± 22^d	145 ± 14^d	$206 \pm 18^{d***}$	$610 \pm 30^{c**}$
MG-200	$682 \pm 39^{a***}$	$265 \pm 20^{c*}$	$151 \pm 16^{b*}$	$214 \pm 15^{c***}$	$630 \pm 23^{b***}$

The values were expressed as pg/mL \pm standard deviation. Yeast glucan (YG; 100, 150, and 200 mg/kg), Oat glucan (OG; 100, 150, and 200 mg/kg), and Mushroom glucan (MG; 100, 150, and 200 mg/kg). *, **, *** indicates the $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively whereas the alphabets *^a-^g represents the significant difference ($p < 0.05$) among the groups.

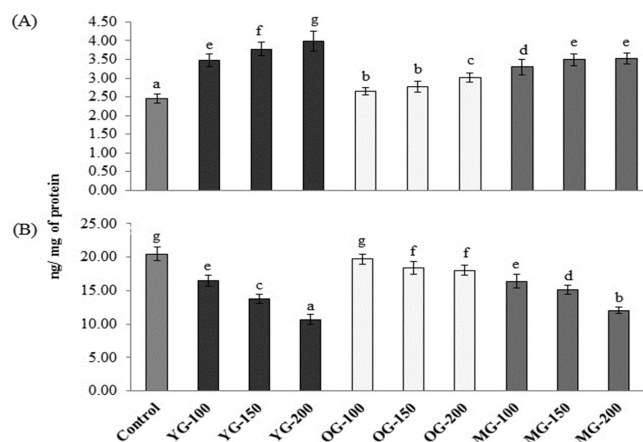


Figure 1. The representation of (A) total antioxidant capacity, in terms of TEAC; and (B) lipid oxidation (MDA assay) in mice liver during experimental supplements. a-g represent the significant difference ($p < 0.05$) among the different groups. YG, OG, and MG indicate that yeast glucan, oat glucan, and mushroom glucan, respectively.

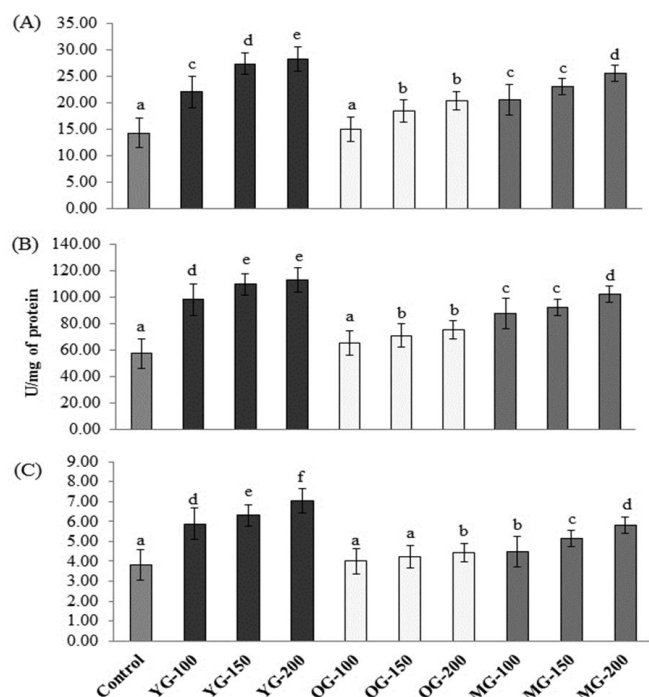


Figure 2. The changes in (A) catalase; (B) superoxide dismutase; and (C) glutathione peroxidase in mice liver during experimental supplements. a-g represent the significant difference ($p < 0.05$) among the different groups. YG, OG, and MG indicate that yeast glucan, oat glucan, and mushroom glucan, respectively.

Oat-BG supplementation nullified the impact of bacterial LPS mediated enteritis, which was characterized by improved values of antioxidative markers, like total antioxidant status, SOD, glutathione reductase and GPx activity in rat colon (Wilczak et al., 2015).

The treatment of BALB/c mice with 50mg/kg/day of sulfated glucans from *S. cerevisiae* for 14 days improved the serum CAT, and GPx activities, and decreased MDA level. The glucan from *S. cerevisiae* showed the *in vitro* scavenging activity in 1,1-Diphenyl-2-picryl-hydrazyl assay, and against superoxide, and hydroxyl radicals (Lei et al., 2015). The chloroform: methanol extract of mushroom, *Polyporus dermatoporus*, was reported for the suppression of 42.9, and 83.3% of lipid peroxidation, and superoxide radicals, respectively in BALB/c mice. Moreover, the anti-inflammatory property of *P. dermatoporus* extract was also observed in terms of the decline in polymorphonuclear cells and nitric oxide, which are further evidenced by histopathological examination of ear edema (Dore et al., 2014).

The total antioxidant capacity of 1 mg of soluble branched β -D-glucan from *Entoloma lividoalbum* was determined to be equal to $70 \pm 15 \mu\text{g}$ of ascorbic acid, and it has also been reported for its hydroxyl and superoxide radical scavenging ability (Maity et al., 2014). β -D-glucan of the mushroom *Russula albonigra* (Krombh.) Fr. has been reported for its antioxidant capacity and nitric oxide mediated macrophage activation, as well as its *in vitro* splenocytes, and thymocytes proliferation (Nandi et al., 2014).

The results of the present study suggest that yeast-BG supplementation at a concentration of 200 mg enhanced the health status of experimental mice by improving the TAC, inhibiting lipid peroxidation, and increasing antioxidant enzymes more than other tested interventions (Figure 1, 2). The anti-colitis property of BG from *S. cerevisiae* HII31 was recently reported by our group. We have also proved that yeast-BG supplementation and rice bran extract (rice phenolic acids, anthocyanins) significantly reduced the consequences of Dextran Sodium Sulphate (DSS) mediated colitis induction in rat model by regulating the serum cytokines, antioxidant capacity and antioxidant enzymes (Pengkumsri et al., 2017b).

4 Conclusion

The data collectively revealed that yeast-BG is a potent activator of inflammatory and anti-inflammatory cytokines, as well as antioxidant capacity of mice than oat-, and mushroom-BG. The bioactivity of BG was directly proportional to the concentration. Moreover, the extraction method plays a major role in the yield of the BG. Thus, a more detailed study is mandatory to explain the influence of extracted BG on the immune system and other biological parameters like antioxidant level variation, which help to develop functional food supplements.

Acknowledgements

The current research was supported by CMU Research grant. All the authors acknowledge the Faculty of Pharmacy, Chiang Mai University, Chiang Mai for the laboratory amenities.

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Appendix A. Supplementary data.

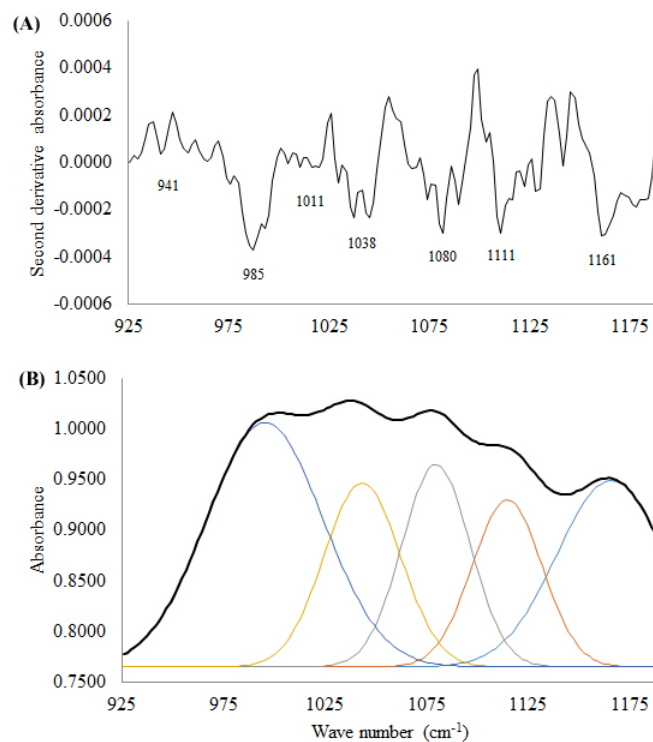


Figure S1. (A) Second derivative of the mean FT-IR spectra of yeast (*S. cerevisiae* HII31) β -glucan; (B) Normalized FT-IR average spectra of yeast (*S. cerevisiae* HII31) β -glucan.

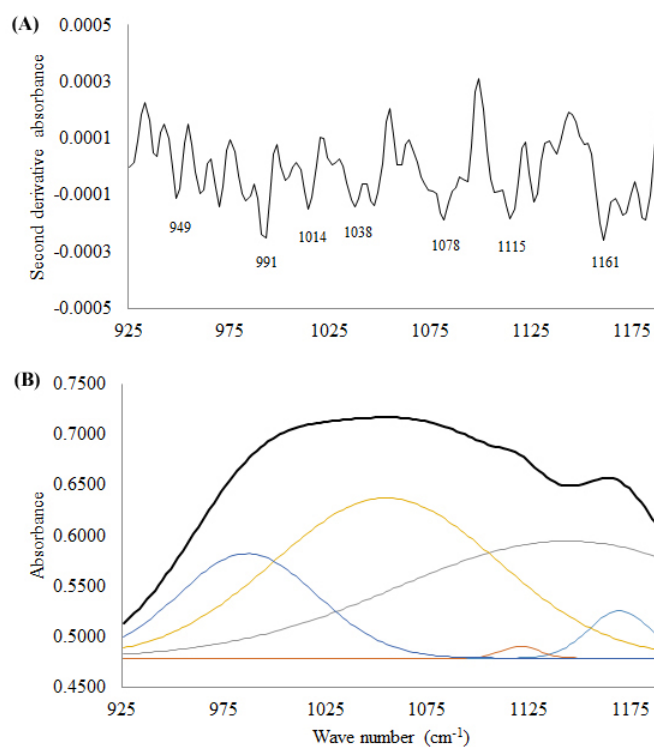


Figure S2. (A) Second derivative of the mean FT-IR spectra of oat (*A. sativa* L.) β -glucan; (B) Normalized FT-IR average spectra of oat (*A. sativa* L.) β -glucan.

Appendix A. Continued...

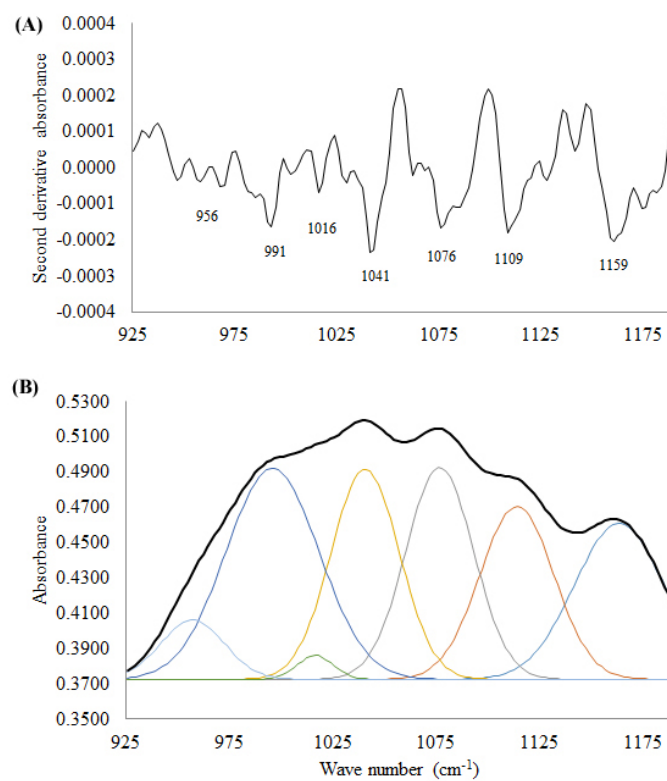


Figure S3. (A) Second derivative of the mean FT-IR spectra of mushroom (*H. erinaceus*) β -glucan; (B) Normalized FT-IR average spectra of mushroom (*H. erinaceus*) β -glucan.