การศึกษาแบบเพิ่มขนาดรับประทานเพื่อกำหนดปริมาณที่ปลอดภัยของเบต้า 1,3/1,6 กลูแคนจากเชื้อราสายพันธุ์ *Ophiocordyceps dipterigena* BCC 2073 ในคนสุขภาพดี

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บทคัดย่อ

เบต้า 1,3/1,6 กลูแคน เป็นส่วนผสมฟังก์ชันในผลิตภัณฑ์เสริมอาหารโดยผลิตจากยีสต์เหลือใช้ในอุตสาหกรรมเบเกอรีจึงมีความบริสุทธิ์ต่ำ ปัจจุบันเทคโนโลยีชีวภาพสมัยใหม่ผลิตเบต้ากลูแคนจากเชื้อราเป็นทางเลือก ข้อดี คือ ปราศจากกลูเตนและมีความบริสุทธิ์สูง แต่เป็นอาหารใหม่ จึงยังไม่ทราบขนาดสูงสุดที่รับประทานได้ปลอดภัย การศึกษาแบบเพิ่มขนาดรับประทานนี้ทำเพื่อศึกษาความปลอดภัยและความทนต่อเบต้า 1,3/1,6 กลูแคนพอลิแซ็กคาไรด์และโอลิโกแซ็กคาไรด์จากเชื้อราสายพันธุ์คอร์ดิเซป ดิปเทอริเจนา บีซีซี 2073 และหาขนาดปลอดภัยสำหรับการบริโภค การศึกษาเพิ่มขนาดแบบรับประทานครั้งเดียวทำในอาสาสมัครสุขภาพดี 12 คน ได้รับแคปซูลเบต้ากลูแคนพอลิแซ็กคาไรด์หรือโอลิโกแซ็กคาไรด์ (กลุ่มละ 6 คน) ขนาด 2-2,000 มิลลิกรัม ขนาดละ 1 ครั้ง แต่ละวันเพิ่มขนาดเป็นขั้นๆ รวม 14 วัน ส่วนการศึกษาเพิ่มขนาดแบบรับประทานหลายครั้งต่อเนื่องอาสาสมัครทั้งหมด 26 คน ได้รับแคปซูลเบต้ากลูแคนพอลิแซ็กคาไรด์ 12 คน หรือโอลิโกแซ็กคาไรด์ 14 คน ขนาด 2-2,000 มิลลิกรัม เป็นขั้นๆ ขั้นละ 2 สัปดาห์ รวม 12 สัปดาห์ วัดผลความปลอดภัยและความทนจากอาการไม่พึงประสงค์ด้วยสมุดบันทึกและค่าเคมีคลินิกในเลือด ผลวิจัยไม่พบอาการไม่พึงประสงค์ร้ายแรง อาการถ่ายบ่อยและถ่ายเหลวพบจากเบต้ากลูแคนพอลิแซ็กคาไรด์ (ร้อยละ 16) มากกว่าโอลิโกแซ็กคาไรด์ (ร้อยละ 7) ค่าเคมีคลินิกปกติตลอดการศึกษา การศึกษานี้ชี้ให้เห็นว่าขนาดสูงสุดของเบต้ากลูแคนจากเชื้อราที่รับประทานในระยะสั้นได้อย่างปลอดภัย คือ 2,000 มิลลิกรัมต่อวัน ในอนาคตควรศึกษาความปลอดภัยระยะยาว

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A Dose-Escalation Study to Evaluate Safety of a Novel β-1 ,3 / 1 ,6 - Glucan from *Ophiocordyceps dipterigena* BCC 2073 Supplementation in Healthy Volunteers

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Abstract

β-1,3/1,6-Glucan, a common functional ingredient in dietary supplements, is usually produced from left-over yeast in the bakery industry with low purity. Owing to gluten-free and high-purity advantages, new cost-effective biotechnology has recently manufactured β-glucan from fungi as an alternative. However, it is a novel food and the safety limit for human consumption is unknown. This dose-escalation study aimed to evaluate the safety and tolerability of β-1,3/1,6-glucan polysaccharides and oligosaccharides from *Ophiocordyceps dipterigena* BCC 2073 in healthy subjects and identify the maximum tolerated dose (MTD). In the single ascending dose phase, 12 participants consumed either β-glucan polysaccharides or oligosaccharides capsules (n = 6 each) one dose for one day in a stepwise dose escalation from 2 to 2,000 mg (total of 14 days). In the multiple ascending dose phase, 26 participants consumed either β-glucan polysaccharides (n = 12) or oligosaccharides (n = 14) one dose daily for 2 weeks in a stepwise escalation from 2 to 2,000 mg (total of 12 weeks). The safety and tolerability were assessed by using subject diaries and blood chemistry. Results show no serious adverse events in all participants throughout the study. More frequent defecation and liquid stool were found in the β-glucan polysaccharide (16%) than in the oligosaccharide group (7%). The average blood biochemical values are all in the normal range. This study suggests that the short-term maximum tolerated dose of β-glucan polysaccharide or oligosaccharide from *O. dipterigena* BCC 2073 is 2,000 mg per day. Further long-term safety trials are warranted.

Keywords: β-Glucan, Fungi, Clinical trial, Safety, Dietary supplements, Dose-escalation study

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Introduction

β-Glucan is a kind of β-D-glucose polysaccharide, widely found in the cell walls of bacteria, fungi, yeasts, algae, mushrooms, lichens, and plants such as oats and barley wheat[1, 2].β-Glucan isolated from different sources varies in the position of glycosidic linkages, degree of branching, molecular weight, solubility, and their effect or function[1, 3, 4]. Of interest, 1, 3/ 1, 6 β-glucan is often found in yeast and fungi and has been known to bind with receptors in immune cells, thus providing an immune-modulating effect[5-7]. The most common source of 1, 3/ 1, 6 β -glucan is from the *Saccharomyces cerevisiae* (*S. cerevisiae*), or baker's yeasts[8]. The European Food Safety Authority (EFSA) defined the maximum limit of yeast β-glucan in food supplements up to 375 mg/ day[9]. Unfortunately, β-glucan isolated from yeast may include some chitin and mannoproteins and may have some restrictions for people with yeast allergies. Furthermore, β-glucan may also be produced from byproduct yeast as waste material in the bakery and beer industry[8]. However, the extraction is quite challenging and yields low purity with a chance of gluten contamination.

New cost-effective biotechnology has recently been performed to extract β-glucan from fungi such as *Ophiocordyceps dipterigena* BCC 2073 to improve purity and provide an alternative for people allergic to gluten or yeast. A fermentation process using specially formulated low-cost media was used to obtain β-glucan polysaccharide. The β-glucan is composed of mannose, galactose, and glucose. The estimated molecular weight was about 800-900 kDa[10, 11]. The structure resembles β-glucan extracted from yeasts with 1, 3/ 1, 6 structure (a (1 / 3) - b-D-glucan backbone substituted at O-6 with side chains of (1/6)-b-D-glucopyranosyl units[12]. The β-glucan polysaccharide was treated by gamma irradiation in a cobalt-60 irradiator to obtain β-glucan oligosaccharides, with a molecular weight of 5 kDa. The smaller size of the oligosaccharide form is thought to increase access to bind with target receptors such as dectin-1 on immune cells[13]. *In vitro* and *in vivo* studies consistently show the effect of β-glucan produced by *O. dipterigena* BCC 2073 as a prebiotic to promote the growth of probiotic bacteria in chicken[11, 14].

β-glucan polysaccharides and oligosaccharides from *O. dipterigena* BCC 2073 are classified as "novel food", as stated by the European Union (EU)'s and Thailand's regulation[15, 16]. Scientific assessment of safety is required before submitting its label to regulatory bodies (such as the European Food Safety Authority (EFSA) or the Thai Food and Drug Administration (Thai FDA), for approval before use. However, the safety limit of this novel β-

glucan for human consumption has never been evaluated.

The previous acute toxicity study of β-glucan polysaccharide from *O. dipterigena* BCC 2073 in Wistar rats reported that the oral LD50 was higher than 15,000 mg/kg body weight[17], and the sub-chronic toxicity study reported that the No-observed-adverse-effect level (NOAEL) of β-glucan polysaccharide was 40 mg/kg body weight/day[18]. Applying an uncertainty factor of 1,000 and an average human body weight of 60 kg, the estimated safe level for human consumption based on animal study is 2.4 mg/ day. The dose is quite low compared to the reported effective dose of 1-3 g/day of β-glucan from other sources[19, 20]. This is due to the ethical limitation in feeding animals defined by the Organisation for Economic Cooperation and Development (OECD)'s guideline No. 408[21]. Therefore, clinical trials are needed to determine the maximum safe levels for human consumption.

This study is the first-in-human trial of β-glucan polysaccharide and β-glucan oligosaccharide from *O. dipterigena* BCC 2073. This dose-escalation study was designed to evaluate the short-term safety and tolerability of the fungal β-glucan in healthy volunteers and identify the maximum tolerated dose (MTD).

Materials and Methods

Ethical aspects and setting

The protocol of this study (MU-CIRB 2022/289.3110) was approved by the Mahidol University Central Institutional Review Board (MU-CIRB), approval number COA. No. MU-CIRB 2022/132.0812. This research was performed according to the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use Good Clinical Practice (ICH-GCP) and the Declaration of Helsinki. Informed written consent was obtained from each participant before the study. The Thai Clinical Trial Registry (TCTR) Committee has reviewed and approved the protocol. The TCTR identification number is TCTR20240705004. The protocol can be accessed at https://www.thaiclinicaltrials.org/show/TCTR20240705004.

Study design

This is a dose-escalation study with a pre-post design. The study comprises a single ascending dose (SAD) phase and a multiple dose (MAD) phase. A single dose of β-glucans was provided once in the SAD phase. Then, the next day the dose had been stepwise escalated from 2 to 2000 mg so the total duration of the study was 14 days. In the MAD phase, participants received a stepwise escalating dose from 2 to 2000 mg of β-glucans once a day. Each dose was

repeated for two weeks so the total duration of the study was 12 weeks. The rationale of using the dose 2-2000 mg/day is based on an animal study and the predicted efficacy. The starting dose of 2 mg/day is from the estimated safe level for human consumption based on sub-chronic toxicity test[18] at 2.4 mg/ day. The dose of 2,000 mg/day was derived from the effective dose of β-glucan around 1,000 mg [19] with two-fold window added for safety purposes.

Participants

The inclusion criteria for screening participants of both the SAD and MAD phases were healthy people aged 18-59 years old with a body mass index no more than 30 kg/m^2, who had no systematic diseases e.g. diabetes mellitus, obesity, liver disease, kidney diseases, thyroid disease, cancer, and autoimmune diseases. The blood tests result within 6 months are in the acceptable normal range: normal complete blood count (CBC) with hemoglobin at least 11 g/ dL, HbA1c ≤ 5.8%, fasting plasma glucose (FPG) ≤ 110 mg/dL, Castelli risk index-I (Total cholesterol (TC)/ high-density lipoprotein cholesterol; (HDL-C) of ≤ 5.0[22], normal liver markers (alanine aminotransferase (ALT), aspartate amino-transferase (AST), bilirubin) and normal kidney function (blood urea nitrogen (BUN), creatinine, and estimated glomerular filtration rate (eGFR). The participants had normal vital signs with blood pressure ≤ 160 / 90 millimeter mercury (mm Hg) and heart rate ≤ 100 beats/min. The exclusion criteria were having COVID-19 infection or exposure to COVID-19-infected patients within 7 days, being unable to come for follow-up, being allergic to fungi or Cordyceps, being pregnant or planning to be pregnant within the next 3 months, breastfeeding, regularly consuming drugs, herbs or dietary supplements, alcohol intake > 14 drinks per week for male or > 7 drinks per week for females and cannot refrain from alcohol intake during the study, smoking > 10 cigarettes/day, having coagulation problems e.g. hemophilia, idiopathic thrombocytopenia, leukemia, in critical condition, and having infectious diseases.

Sample size

The sample size of this study utilized the same number as previous dose-escalation studies for dietary supplements[23-24]. For the SAD phase, 12 participants were randomly allocated into β-glucans polysaccharide and oligosaccharide groups (n = 6, each). For the MAD phase, 24 participants were randomly allocated into β-glucan polysaccharide and oligosaccharide groups (n = 12, each). The rationale for the sample size of 6 and 12 comes from the 3+3 principle for clinical safety assessment (phase I clinical trial) to define the MTD.

The 3+3 principle states that if at least one subject among the total subjects of three experienced adverse events, three more subjects should be added to the study[25-26]. MTD is the dose below the dose at which >33% of participants reported adverse events. Thus, for the SAD phase if at least 3 out of 6 participants had adverse events (dose-limiting toxicities), the study would be stopped, and the dose below that dose would be the MTD. For the MAD phase, the MTD was defined as the dose below the dose at which at least 5 out of 12 participants had adverse events[23, 24].

Materials

β-glucans polysaccharide and β-glucans oligosaccharide were provided by Asia Star Trade Co., Ltd. Bangkok, Thailand. The product is in capsule form. The production process starts with fermentation using *O. dipterigena* BCC 2073 with a specially optimized media to obtain β-glucans polysaccharide (about 900 kDa of molecular weight). Then, the β-glucans polysaccharide was treated by 100 kGy gamma irradiation in a cobalt-60 irradiator to obtain β-glucans oligosaccharides (about 5-8 kDa of MW). After that, β-glucans were dried by a drum dryer, crushed into powder, and filled into capsules of size No.0 according to supplementation dose. Both polysaccharide and oligosaccharide products contain β-glucans of at least 85%. Other components include fat, protein, moisture, ash, potassium, phosphorus, calcium, magnesium, sulfur, and other minerals in trace amounts. The products passed the safety standard requirement of the Food and Drug Administration of Thailand (microbiological and chemical contaminant testing) according to the Notification of the Ministry of Public Health No.293, 309, 405, 411, and 416[27-31]. Cadmium (mg/kg), Lead (mg/kg), and Mercury (mg/kg) were not detected. Arsenic is less than 0.185 mg/kg for both products. *Clostridium* spp., *Salmonella* spp., and *Staphylococcus aureus* were not detected in 0.1 g, 25 g, and 0.1 g, respectively. *Escherichia coli* was less than 3 MPN/ g. Yeasts and Molds were less than 10 colony forming units (cfu)/ g for both products. It is worth noting that all products used in this study were produced in the same manufacturing batch.

Intervention

In these trials, β-glucans capsules (β-glucans polysaccharide or β-glucans oligosaccharide) were given to participants in a dose-escalation manner. In the SAD phase, participants received one dose for one day in step-wise escalation from 2 to 2,000 mg of β-glucans polysaccharide or β-glucans oligosaccharide. Each assigned dose was given in one sachet as shown in

Figure 1. For MAD phase, participants consumed one dose daily for two weeks in a step-wise escalation of 2, 100, 500, 1,000, 1,500, and 2,000 mg. Each assigned dose was given in one sachet as shown in Figure 2.

Days	Administration dose (mg)	Number of capsules	Days	Administration dose (mg)	Number of capsules
1	2	Capsules contain 2 mg, 1 capsule 	8	1100	Capsules contain 500 mg, 2 capsules and 100 mg, 1 capsule 
2	50	Capsules contain 50 mg, 1 capsule 	9	1300	Capsules contain 500 mg, 2 capsules and 300 mg, 1 capsule 
3	100	Capsules contain 100 mg, 1 capsule 	10	1500	Capsules contain 500 mg, 3 capsules 
4	300	Capsules contain 300 mg, 1 capsule 	11	1700	Capsules contain 500 mg, 3 capsules and 200 mg, 1 capsule 
5	500	Capsules contain 500 mg, 1 capsule 	12	1800	Capsules contain 500 mg, 3 capsules and 300 mg, 1 capsule 
6	700	Capsules contain 500 mg, 1 capsule and 200 mg, 1 capsule 	13	1900	Capsules contain 500 mg, 3 capsules and 400 mg, 1 capsule 
7	900	Capsules contain 500 mg, 1 capsule and 400 mg, 1 capsule 	14	2000	Capsules contain 500 mg, 4 capsules 

Figure 1. Diagram of intervention for single ascending dose (SAD) phase

The diagram shows the size and number of capsules for each dose. During this phase, the participant consumed each assigned dose once daily for one day and stepwise escalated to a higher dose as shown.

Study procedure and outcome measurement

The monocentric dose-escalation study was performed on healthy participants. Dose escalation was carried out in two phases: single ascending dose (SAD), and multiple ascending (MAD) phases.

- ***Single ascending dose (SAD)***

As shown in Figure 3, there were 3 visits. In the first visit (V1), recruited volunteers were screened according to the inclusion and exclusion criteria. History taking for medical status and medication/dietary supplement use were performed. Vital signs (blood pressure, and heart rate), body weight, and blood biochemical parameters (complete blood count, fasting blood glucose, lipid profile, liver, and kidney function biomarkers) were

measured. In the second visit (V2), β-glucans capsules were given to participants in each group with instructions to consume one assigned dose for a day and recorded in a subject diary (adverse events, product intake, and consumption of other food). After taking the first dose, if no adverse events or adverse effects occurred in no more than 2 subjects, participants could step up to the higher assigned dose until the end of the study. However, if at least 3 persons had adverse symptoms at any dose, the study would be terminated. The MTD is the previous safe dose level. The number of adverse effects was determined by using both the subject diary for a daily self-record of adverse symptoms by the participants and a daily telephone follow-up by the researcher. All participants were informed to continue the next dose if they had no symptoms for each dose. If there were any symptoms, they were asked to report to the researcher and discuss whether to continue or terminate the intervention. Throughout the study, the participants were asked to avoid taking any dietary supplements, and alcoholic beverages. They were asked to call us immediately when they had any serious abnormal symptoms. The SAD started at 2 mg and escalated to 50, 100, 300, 500, 700, 900, 1,100, 1,300, 1,500, 1,700, 1,800, 1,900, and 2,000 mg. After 14 days of dose-escalated intervention, in the last visit (V3) all participants came for a follow-up of adverse symptoms and returned the empty sachets for a compliance check. Body weight and vital signs were measured.

Weeks	Administration dose (mg)	Number of capsules	
1-2	2	Capsules contain 2 mg, 1 capsule	
3-4	100	Capsules contain 100 mg, 1 capsule	
5-6	500	Capsules contain 500 mg, 1 capsule	
7-8	1000	Capsules contain 500 mg, 2 capsules	
9-10	1500	Capsules contain 500 mg, 3 capsules	
11-12	2000	Capsules contain 500 mg, 4 capsules	

Figure 2. Diagram of intervention for multiple ascending dose (MAD) phase

The diagram shows the size and number of capsules for each dose. During this phase, the participant consumed each assigned dose once daily for two weeks and stepwise escalated to a higher dose as shown.

Visit 1 Screening: collection of demographic parameters (age, body weight, BMI), vital signs, blood biochemical values

Screening period 4-7 days

Visit 2 Product delivery: Issues of Beta- Glucan orally ingested capsules (Beta-Glucan polysaccharide and Beta-Glucan oligosaccharide groups) and subject diaries

Administration period: Subject receive Beta- Glucan orally ingested capsules (Beta- Glucan polysaccharide and Beta-Glucan oligosaccharide groups) in following dosages & timeframes

Duration (days)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Dose (mg)	2	50	100	300	500	700	900	1100	1300	1500	1700	1800	1900	2000
Serving size (Capsule No.0)	1 (2 mg=1)	1 (50 mg=1)	1 (100 mg=1)	1 (300 mg=1)	1 (500 mg=1)	2 (500 mg=1, 200 mg=1)	2 (500 mg=1, 400 mg=1)	3 (500 mg=2, 100 mg=1)	3 (500 mg=2, 300 mg=1)	3 (500 mg=3)	4 (500 mg=3, 200 mg=1)	4 (500 mg=3, 300 mg=1)	4 (500 mg=3, 400 mg=1)	4 (500 mg=4)
Frequency	Once daily	Once daily	Once daily	Once daily	Once daily	Once daily	Once daily	Once daily	Once daily	Once daily	Once daily	Once daily	Once daily	Once daily
Total number subjects	P = 6 O = 6	P = 6 O = 6	P = 6 O = 6	P = 6 O = 6	P = 6 O = 6	P = 6 O = 6	P = 6 O = 6	P = 6 O = 6	P = 6 O = 6	P = 6 O = 6	P = 6 O = 6	P = 6 O = 6	P = 6 O = 6	P = 6 O = 6
0-2 subjects with adverse events	Escalation to next dose													MTD is 2000 mg
≥3 subjects with adverse events	Stop	Stop MTD is 2 mg	Stop MTD is 50 mg	Stop MTD is 100 mg	Stop MTD is 300 mg	Stop MTD is 500 mg	Stop MTD is 700 mg	Stop MTD is 900 mg	Stop MTD is 1100 mg	Stop MTD is 1300 mg	Stop MTD is 1500 mg	Stop MTD is 1700 mg	Stop MTD is 1800 mg	Stop MTD is 1900 mg

Visit 3 Follow-up after 14 days: Collection of subject diaries, interview for adverse events, body weight, and vital signs

Figure 3. The study flow scheme of the single ascending dose (SAD) phase

BMI, body mass index; P, β-glucans polysaccharide; O, β-glucans oligosaccharides; MTD, maximum tolerated dose

- ***Multiple ascending doses (MAD)***

After the SAD phase was completed, repeated doses for the MAD phase were selected based on the SAD phase. As shown in Figure 4, there were 8 visits. In the first visit (V1), recruited volunteers were screened. Vital signs (blood pressure, and heart rate), body weight, and blood biochemical parameters were measured as baseline values. In the second visit (V2), β-glucans capsules were given to participants in each group with instructions to consume each assigned dose once a day for two weeks and recorded in a subject diary (adverse events, product intake, and consumption of other food). For dietary records, they were asked to record food they consumed 3 days per week (two weekdays and one weekend). After taking the first dose, if there were no adverse events or adverse effects in no more than 4 subjects, participants could step up to the higher assigned dose until the end of the study. However, the study would be terminated if at least 5 subjects had adverse symptoms. The MTD is the previous safe dose level. Throughout the study, the participants were asked to avoid taking any

dietary supplements, and alcoholic beverages. They were asked to call the researchers immediately when they had any serious abnormal symptoms. In visits 3-8 (V3-V8), participants returned for follow-up of adverse symptoms and returned the empty sachets for a compliance check. Body weight, vital signs, and blood biochemical parameters were measured and compared with the baseline. The MAD started at the dose of 2 mg once daily repeatedly for two weeks and escalated to 100, 500, 1,000, 1,500, and 2,000 mg once daily repeated for two weeks each. The total study duration was 12 weeks.

Visit 1 Screening: collection of demographic parameters (age, body weight, BMI), vital signs, blood biochemical values

Screening period 4-7 days

Visit 2 Product delivery: Issues of β-glucan orally ingested capsules (β-glucan polysaccharide and β-glucan oligosaccharide groups) and subject diaries

Administration and Data collection scheme						
Dose (mg)	2	100	500	1000	1500	2000
Serving size (Capsule No.0)	1 (2 mg=1)	1 (100 mg=1)	1 (500 mg=1)	2 (500 mg=2)	3 (500 mg=3)	4 (500 mg=4)
Total number subjects	P = 12 O = 12	P = 12 O = 12	P = 12 O = 12	P = 12 O = 12	P = 12 O = 12	P = 12 O = 12
0-4 subjects with adverse events						MTD is 2000 mg/d
≥5 subjects with adverse events	Stop	Stop MTD is 2 mg/d	Stop MTD is 100 mg/d	Stop MTD is 500 mg/d	Stop MTD is 1100 mg/d	Stop MTD is 1500 mg/d

Visit 3-8 Follow-up: Collection of subject diaries, interview for adverse events, body weight, vital signs, and blood biochemical values

Figure 4. The study flow scheme of the multiple ascending doses (MAD) phase

BMI, body mass index; P, β-glucans polysaccharide; O, β-glucans oligosaccharides; MTD, maximum tolerated dose

Data analysis

Characteristics of participants were summarized as mean and standard deviation (SD) for numerical data, and frequency and percent for categorical data. Adverse events were counted and displayed as percentages. This study used intention-to-treat analysis; i.e. all data of all participants were used for analysis. Diet analysis of dietary records was performed by using INMUCAL-Nutrient V.4.0 (Institute of Nutrition, Mahidol University. Phutthamonthon, Nakhon Pa-

thom, Thailand). The body weight and blood biochemical parameters after consuming doses of β-glucans capsules (β-glucans polysaccharide and β-glucans oligosaccharide) were compared with baseline by using the Kruskal - Wallis test, followed by Dunn's multiple comparison test. Changes in dietary intake throughout the study were analyzed using the Friedman test followed by Dunnett's multiple comparison test. Statistical tests were performed by using a two-tailed test. P-value < 0.05 was considered statistically significant. Graph Pad Prism V.9.3 (GraphPad Software, Boston, Massachusetts, USA) was used for graphing and statistical analyses.

Results

Single ascending dose (SAD)

- ***Baseline characteristics***

As shown in Table 1, a total of 12 healthy participants (n=6 for each group) were randomly assigned to β-glucans polysaccharide groups (2 males and 4 females) and β-glucans oligosaccharide groups (2 males and 4 females). The average age of β-glucans polysaccharide and oligosaccharide groups were 30.83 ± 5.79 years old, and 35.50 ± 11.93 years old, respectively. The mean BMI of the polysaccharide and oligosaccharide groups were 23.00 ± 4.56 kg/m^2, and 23.17 ± 3.60 kg/m^2, respectively. All subjects had no histories of past and present illness, smoking, or drinking. All subjects completed the study with no drop-out.

Table 1. Demographic characteristics of participants in single ascending dose (SAD) phase

Parameter	β-glucans polysaccharide (n=6)	β-glucans oligosaccharide (n=6)
Gender, n (%)		
Male	2 (33)	2 (33)
Female	4 (67)	4 (67)
Age (years)		
Mean ± SD	30.83 ± 5.79	35.50 ± 11.93
Range (min-max)	23-40	25-59
Body mass index (BMI (kg/m^2)		
Mean ± SD	23.00 ± 4.56	23.17 ± 3.60
Range (min-max)	18-30	19-29
Systemic diseases, n (%)	0 (0)	0 (0)
Smoking, n (%)	0 (0)	0 (0)
Alcohol drinking, n (%)	0 (0)	0 (0)

- ***Adverse event and maximum tolerated dose***

As displayed in Table 2, minor adverse events occurred in 2 out of 6 subjects consuming 1,100 mg β-glucan polysaccharide. No adverse events were observed in the oligosaccharide group. As shown in Table 3, the minor adverse symptom was mild abdominal discomfort (pain score of 1 out of 10), which was tolerable and no treatments were required. The discomfort was not dose-dependent since the stepwise dose escalation did not worsen the symptom. Interestingly, a participant in the oligosaccharide group reported improved bowel movements with normal stool characteristics. The event was considered beneficial for the participants. Throughout the study, there were no serious adverse events in both polysaccharide and oligosaccharide groups. Thus, the MTD of β-glucans polysaccharide and oligosaccharide capsules taken orally for a single intake was 2,000 mg.

Multiple ascending doses (MAD)

- ***Baseline characteristics***

As shown in Table 4, a total of 26 healthy participants were randomly assigned to β-glucans polysaccharide groups (n = 12, 4 males and 8 females) and β-glucans oligosaccharide groups (n = 14, 5 males and 9 females). The average age of β-glucans polysaccharide and oligosaccharide groups was 36.83 ± 7.72 years old and 36.86 ± 9.37 years old, respectively. The mean BMI of β-glucans polysaccharide and oligosaccharide groups was 24.15 ± 3.21 kg/m^2, and 23.27 ± 4.04 kg/m^2, respectively. All subjects had no histories of past and present illness, smoking, or drinking. As described in Figure 5, during the study, four subjects withdrew from the study (1 in the polysaccharide group and 3 in the oligosaccharide group). The reasons for drop-out were COVID-19 infection (n = 2), diarrhea (history of gastritis) (n = 1), and nail surgery with prescribed antibiotic drug intake (n = 1).

- ***Adverse event***

As shown in Tables 5-6, more frequent bowel movement was found in 8-16 % at the dose of 100-2,000 mg β-glucans polysaccharide. Liquid stool was found in 8% of participants consuming 2,000 mg polysaccharide. Among participants consuming 2 - 1,000 mg oligosaccharide, 7-21% had liquid stool and 7-14% had gas in the gastrointestinal tract. All adverse symptoms were minor, transient, and tolerable, no treatments were required.

- ***Body weight change***

During the MAD phase, there were no relevant significant changes in body weight observed between the β-glucans polysaccharide group and the β-glucans oligosaccharide group intake (Figure 6).

Table 2. Summary of adverse events in the single ascending dose (SAD) phase

Variables	β-glucans polysaccharide (n = 6) (2-2000 mg/day) N, (%),	β-glucans oligosaccharide (n = 6) (2-2000 mg/day) N, (%)
Subjects with any adverse events	2 (33.33)	0
Specific adverse events		
Nausea	0	0
Vomiting	0	0
Abdominal pain	0	0
Abdominal discomfort	2 (33.33)	0
Gas in the gastrointestinal tract	0	0
Headache	0	0

Table 3. Minor gastrointestinal symptoms during β-glucans polysaccharide intake in SAD phase

Subject	Dosage (mg/day)	Details
Subject No. 1	300 700 900 1,100	Abdominal discomfort after β-glucans polysaccharide intake for 30 minutes, rating the degrees on the abdominal pain scale of level 1 from level 10
Subject No. 2	1,100 1,300 1,500 1,700	Abdominal discomfort after β-glucans polysaccharide intake for 24 hours, rating the degrees on the abdominal pain scale of level 1 from level 10

- ***Changes in clinical chemistry and hematology parameters***

Throughout the study, after consuming 2 - 2,000 mg β-glucans oligosaccharide or polysaccharide**,** there were no significant changes in total cholesterol, triglycerides, HDL, BUN, AST, ALT, and all CBC parameters (Table 7 - 8). For the β-glucans oligosaccharide groups, the average fasting blood glucose levels were significantly decreased ($p < 0.05$), compared to baseline values after consum-ing the product at a dose of 2 mg /day (Table 8). However, when escalating to the higher dose, the fasting blood glucose levels tend to increase compared to baseline values, but not significantly. Throughout the study, the blood sugar values were still in a normal

range. For the β-glucans polysaccharide group, the average fasting blood glucose levels were increased compared to baseline values after an intake dose of 100-2,000 mg /day with statistical significance at the dose of 2,000 mg/day ($p < 0.05$). However, the increased values were in a normal range, suggesting no toxicities. LDL levels tend to decrease gradually when the doses of polysaccharide or oligosaccharide are escalated. A significant decrease was seen for 1,500-2,000 mg/day of polysaccharide ($p < 0.05$). Creatinine levels tend to increase gradually when the doses of polysaccharide or oligosaccharide are escalated. A significant decrease was seen for 2,000 mg/day of polysaccharide ($p < 0.05$). eGFR tends to increase gradually when the doses of polysaccharide or oligosaccharide are escalated. A significant decrease was seen for 1,000-2,000 mg/day of polysaccharide ($p < 0.05$). However, all changes in creatinine and eGFR were in a normal range, suggesting no toxicities.

Table 4. Demographic characteristics of multiple ascending dose (MAD) phase

Parameter	β-glucans polysaccharide (n = 12)	β-glucans oligosaccharide (n = 14)
Gender, n (%)		
Male	4 (33)	5 (36)
Female	8 (67)	9 (64)
Age (years)		
Mean ± SD	36.83 ± 8.07	36.86 ± 10.05
Range (min-max)	29-49	23-59
Body mass index (kg/m^2)		
Mean ± SD	24.15 ± 3.35	23.27 ± 4.12
Range (min-max)	19-29	18-30
Illness, n (%)	0 (0)	0 (0)
Smoking, n (%)	0 (0)	0 (0)
Alcohol drinking, n (%)	0 (0)	0 (0)

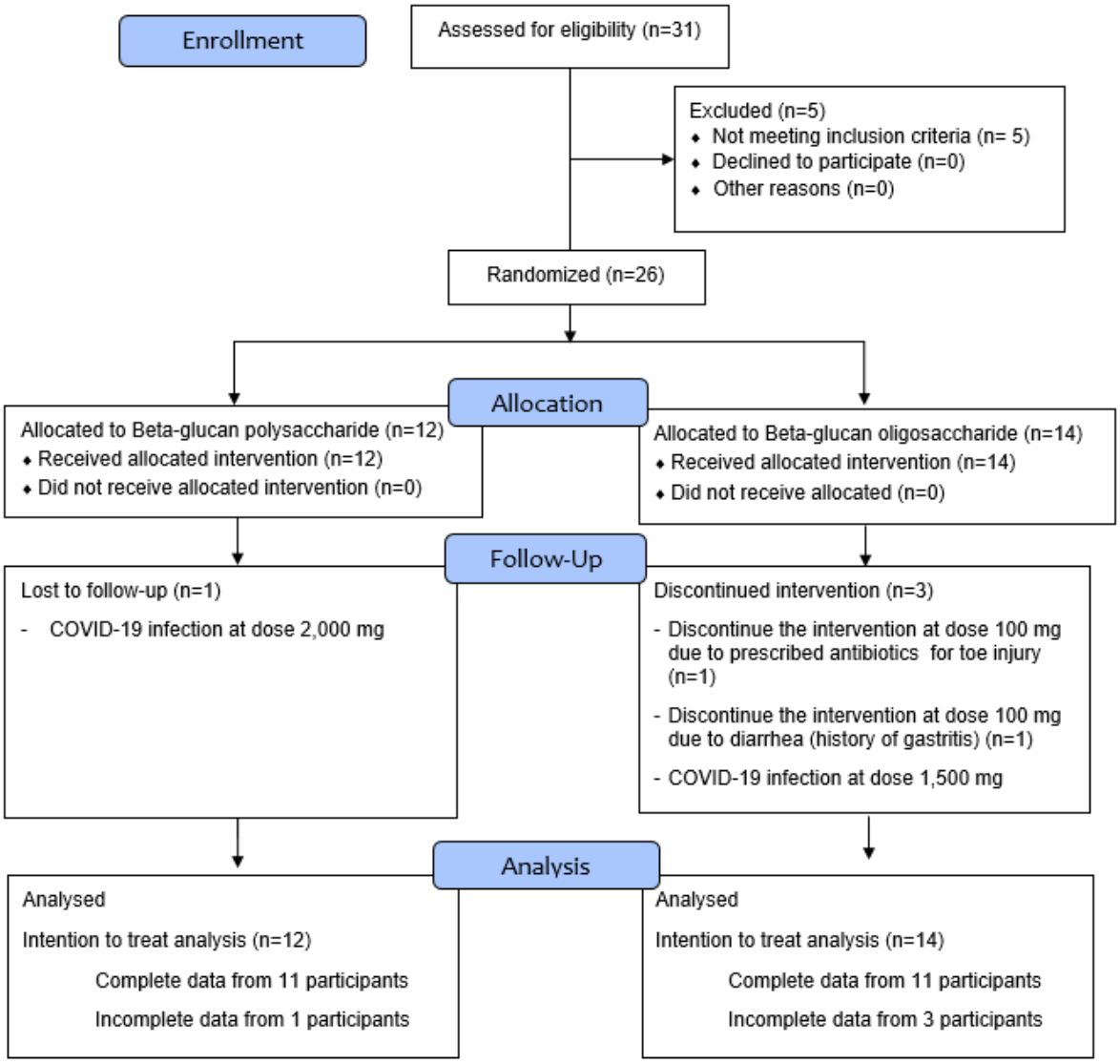


Figure 5. Participants flow diagram of multiple ascending doses (MAD) phase

BMI, body mass index; P, β-glucans polysaccharide; O, β-glucans oligosaccharides; MTD, maximum tolerated dose

- ***Dietary intake during the study***

As shown in Figures 7A-B, energy, and carbohydrate intakes were significantly higher at 2,000 mg/day of β-glucans polysaccharide, compared to those of 2 mg/day. Moreover, there was a trend of decreased dietary fiber intake (Figure 7C), while the sugar intake remained similar throughout the study (Figure 7D). Fat intake was increased at a dose of 2,000 mg/day. Also, protein intakes were gradually increased during the doses of 1,000-2,000 mg/day of β-glucans polysaccharide, but these changes were not statistically significant (Figure 7E-F).

As shown in Figure 8, energy, carbohydrates, fat, dietary fiber, and protein intakes at 1,500 mg/day of β-glucans oligo-

saccharide were notably higher than other dosages. However, the changes were not dose-dependent and not statistically significant (Figures 8A-C, and 8E-F). In addition, sugar intakes during 1,000-2,000 mg/day of β-glucans oligosaccharide were higher than those of 2 mg β-glucans oligosaccharide. However, the differences were not significant (Figure 8D).

It is worth noting that the data of blood glucose and lipid profile on Tables 7-8 showed that there are no significant changes in those parameters at the dose of 100 and 500 mg/ day. Therefore, there is no need to present data of energy and nutrient intake at these data points.

Table 5. Adverse events reported by the subjects in β-glucans polysaccharide group in MAD phase (n = 12)

Dosage (mg/day)	Adverse events symptoms	N, (%)	Details
2	No adverse events		
100	More frequent bowel movement	1, (8)	Increased from 1 time/ day to 2 times/ day, normal stool, no diarrhea
500	More frequent bowel movement	2, (16)	Subject 1: Increased from 1 time/day to 2 times/day, liquid stool, no abdominal pain Subject 2: Increased from 1 time/day to 2 times/day on the 3rd, 5th, and 6th day after taking, normal stool, no diarrhea
1,000	More frequent bowel movement	1, (8)	Increased from 1 time/day to 2 times/day, normal stool, no diarrhea
1,500	More frequent bowel movement	1, (8)	Increased from 1 time/day to 2 times/day, normal stool, no diarrhea
2,000	More frequent bowel movement	1, (8)	Increased from 1 time/day to 2 times/day, normal stool, no diarrhea
	Transient liquid stool	1, (8)	Liquid stool for 2 days, no severe abdominal pain, no medications

- ***Maximum Tolerated Dose (MTD) for repeated intake***

Since the percentage of minor adverse events of all doses in both groups did not exceed 33% and the altered blood parameters were still in the normal range, the MTD of β-glucans polysaccharide and β-glucans oligosaccharide can be concluded at the highest dose of this study, which is 2,000 mg per day.

Table 6. Adverse events reported by the subjects in β-glucans oligosaccharide group in MAD phase (n =14)

Dosage (mg/day)	Adverse events symptoms	N, (%)	Detail
2	Transient liquid stool	1, (7)	Liquid stool on the 1st day after sample taking.
100	Transient liquid stool	3, (21)	Subject 1: Slight liquid stool on 1st day, more liquid stool on 2nd day, abdominal discomfort on 3rd day in the morning, no diarrhea Subject 2: Liquid stool 2 times with no abdominal discomfort when taken 10 minutes before consuming olive oil (no symptoms on other days), no diarrhea Subject 3: Liquid stool with no abdominal discomfort when taken 15 minutes before eating, no diarrhea
	Transient abdominal discomfort	1, (7)	Abdominal discomfort when taking 15-20 minutes before drinking milk
500	Liquid stool	1, (7)	Transient liquid stool, no abdominal pain
1,000	Liquid stool	1, (7)	Liquid stool for 4 days, no abdominal pain
	More gas in the gastrointestinal tract and abdominal discomfort	2, (14)	Subject 1: More gas in the gastrointestinal tract, no abdominal pain Subject 2: Slight abdominal discomfort 30 minutes after taking (occur twice)
1,500	No adverse events		
2,000	No adverse events		

Table 7. Changes in blood parameters after supplementation of 2-2,000 mg/day of β-glucans polysaccharide in MAD phase

Parameter (Unit)	Normal rage	Baseline	2 mg/day	100 mg/day	500 mg/day	1000 mg/day	1500 mg/day	2000 mg/day
Fasting blood glucose (mg/dL)	70-110	89.83±6.45	82.58±7.33	95.25±5.82	96.17±4.45	94.33±4.42	97.18±4.71	100.4±6.55**
Total cholesterol (mg/dL)	<200	212.40±21.65	209.90±25.09	206.30±16.01	201.00±24.52	197.50±27.96	193.70±26.44	200.10±20.52
Triglycerides (mg/dL)	<150	91.58±18.29	90.33± 21.01	100.8±23.90	106.2±33.99	100.6±26.22	108.0±25.33	107.8±28.06
HDL cholesterol (mg/dL)	>45	63.50±19.91	64.25±18.10	66.42±17.20	66.58±19.90	65.67±16.63	67.09±21.20	69.67±20.16
LDL cholesterol (mg/dL)	<130	130.50±19.51	127.60±21.12	119.90±19.95	113.30±22.45	113.60±21.61	105.10±16.44*	108.80±17.52*
BUN (mg/dL)	6-20	12.00±2.92	12.83±2.59	10.83±1.59	10.50±1.78	12.08±1.93	11.64±2.69	11.45±2.07
Creatinine (mg/dL)	0.5-1.5	0.68±0.06	0.70±0.12	0.70±0.12	0.66±0.14	0.83±0.16	0.83±0.15	0.89±0.18*
eGFR (ml/min/1.73 m^2)	>90	115.90±7.69	114.80±6.12	114.80±6.12	117.50±6.84	100.70±10.30**	103.40±10.52*	100.30±12.05**
AST (U/L)	0-40	16.67±6.43	20.67±7.02	14.67±2.52	14.00±0.00	14.33±2.89	21.00±5.29	17.00±2.00
ALT (U/L)	0-40	19.25±5.56	17.00±4.47	18.25±7.24	17.50±5.81	16.83±5.37	20.27±5.24	20.73±7.09
WBC (cells/cu.mm)	4,000-10,000	6.55±0.95	6.71±1.14	6.24±2.11	6.23±1.08	6.73±1.41	6.77±0.98	6.43±1.05
RBC (million cells/cu.mm)	Male: 4.5–6.0, Female: 4.0–5.5	4.70±0.50	4.83±0.53	4.79±0.57	4.75±0.51	4.79±0.53	4.78±0.46	4.76±0.48
Hemoglobin (g/dL)	Male: 13.0–18.0, Female: 12.0–16.0	13.24±1.43	13.51±1.28	13.48±1.39	13.45±1.27	13.47±1.38	13.55±1.24	13.52±1.31
Hematocrit (%)	Male: 40-54, Famale: 36–58	40.38±3.92	41.33±3.58	41.58±3.63	41.08±3.29	40.83±3.71	41.09±3.30	40.82±3.46
MCV (fL)	80.0–99.0	84.41±4.76	85.04±5.06	86.49±5.82	84.75±5.12	84.61±4.54	85.07±4.63	85.06±4.15
MCH (pg)	27.0–31.0	27.98±1.71	27.86±1.85	28.03±1.63	27.86±1.90	27.94±1.85	28.15±1.46	28.2±1.58
MCHC (g/dL)	33.0–37.0	33.13±0.74	32.77±0.88	32.49±1.74	32.69±0.55	33.03±0.77	33.10±0.67	33.14±0.69
RDW (%)	11.0–14.5	13.14±0.83	13.21±0.77	14.01±3.32	13.41±0.88	13.07±0.90	12.98±0.81	13.10±0.91
Platelet Count (cells/cu.mm)	140,000–450,000	281,750.00 ±45,191.00	288,167.00 ±43,419.00	278,167.00 ±48,707.00	280,917.00 ±41,645.00	274,500.00 ±44,574.00	267,182.00 ±44,102.00	254,182.00 ±57,661.00

Data are shown as mean ± SD, * and ** represent $p < 0.05$ and $p < 0.01$, respectively, compared to baseline values .HDL, high-density lipoprotein; LDL, low-density lipoprotein; BUN, Blood Urea Nitrogen; eGFR, estimated Glomerular Filtration Rate; AST, Aspartate transaminase; ALT, Ala-nine aminotransferase; WBC, White blood cell count; RBC, Red blood cell count; MCV, Mean cell volume; MCH, Mean cell hemoglobin; MCHC, Mean cell hemoglobin concentration; RDW, Red blood cell distribution width.

Table 8. Changes in blood parameters after supplementation of 2-2,000 mg/day of β-glucans oligosaccharide in MAD phase

Parameter (Unit)	Normal rage	Baseline	2 mg/day	100 mg/day	500 mg/day	1000 mg/day	1500 mg/day	2000 mg/day
Fasting blood glucose (mg/dL)	70-110	97.57±7.24	89.71±7.03*	92.17±7.20	93.33±6.89	90.92±6.78	96.75±5.74	101.80±6.27
Total cholesterol (mg/dL)	<200	212.60±33.26	216.60±28.73	213.00±36.33	216.70±32.14	214.60±30.65	210.00±25.38	216.00±31.75
Triglycerides (mg/dL)	<150	89.23 ±30.89	92.00 ±34.21	89.45±20.53	88.36±16.39	84.45±17.70	93.45±23.49	96.11±14.18
HDL cholesterol (mg/dL)	>45	64.07±14.42	64.14±12.54	62.58±9.58	64.33±10.31	66.50±13.24	67.50±10.41	65.91±9.18
LDL cholesterol (mg/dL)	<130	130.10±32.57	134.00±27.34	128.20±27.91	132.10±31.13	122.80±23.86	120.00±25.38	127.30±27.46
BUN (mg/dL)	6-20	13.21±3.07	12.71±4.05	13.00±3.52	13.17±4.20	12.58±3.50	12.33±3.42	12.18±3.84
Creatinine (mg/dL)	0.5-1.5	0.76±0.12	0.74±0.11	0.73±0.14	0.73±0.11	0.80±0.17	0.83±0.13	0.84±0.15
eGFR (ml/min/1.73 m^2)	>90	110.60±10.30	112.20±11.61	112.50±17.65	112.30±12.23	106.90±12.63	100.20±13.26	101.70±13.25
AST (U/L)	0-40	17.21±3.40	18.57±4.62	16.25±4.73	16.42±2.50	17.58±3.85	17.75±5.05	17.00±3.94
ALT (U/L)	0-40	18.07±3.97	18.29±6.26	19.67±6.95	17.67±5.76	18.92±6.42	21.33±8.93	18.90±7.30
WBC (cells/cu.mm)	4,000-10,000	6.30±1.79	5.69±1.10	6.29±1.04	6.35±1.27	6.33±1.55	6.81±1.79	6.49±1.57
RBC (million cells/cu.mm)	Male: 4.5–6.0, Female: 4.0–5.5	4.68±0.38	4.69±0.44	4.66±0.45	4.71±0.43	4.66±0.4	4.76±0.35	4.76±0.4
Hemoglobin (g/dL)	Male: 13.0–18.0, Female: 12.0–16.0	12.96±1.41	13.06±1.49	12.79±1.24	12.92±1.15	12.85±1.24	13.10±1.04	13.14±1.39
Hematocrit (%)	Male: 40-54, Famale: 36–58	40.07±4.01	40.29±4.12	39.75±3.47	40.00±3.49	39.75±3.42	40.75±2.80	40.60±3.34
MCV (fL)	80.0–99.0	84.53±4.07	84.96±4.28	84.61±4.02	84.33±4.47	84.23±4.15	84.63±4.08	84.65±3.50
MCH (pg)	27.0–31.0	27.41±1.72	27.56±1.83	27.26±1.87	27.28±1.76	27.33±1.89	27.26±1.72	27.41±1.62
MCHC (g/dL)	33.0–37.0	32.42±0.70	32.42±0.80	32.20±0.97	32.18±0.68	32.43±0.93	32.19±0.82	32.37±1.07
RDW (%)	11.0–14.5	13.39±1.08	13.41±0.90	13.29±0.84	13.38±1.00	13.30±1.15	13.38±1.37	13.35±1.14
Platelet Count (cells/cu.mm)	140,000–450,000	285,286.00 ±59,509.00	281,857.00 ±55,741.00	266,917.00 ±51,352.00	272,750.00 ±62,122.00	264,667.00 ±69,753.00	258,500.00 ±60,916.00	260,400.00 ±61,886.00

Data are shown as mean ± SD and * represent $p < 0.05$ and $p < 0.01$, respectively, compared to baseline values .HDL, high-density lipoprotein; LDL, low-density lipoprotein; BUN, Blood Urea Nitrogen; eGFR, estimated Glomerular Filtration Rate; AST, Aspartate transaminase; ALT, Ala-nine aminotransferase; WBC, White blood cell count; RBC, Red blood cell count; MCV, Mean cell volume; MCH, Mean cell hemoglobin; MCHC, Mean cell hemoglobin concentration; RDW, Red blood cell distribution width

Discussion

β-Glucan polysaccharide and oligosaccharide from *O. dipterigena* BCC 2073 are considered novel food, requiring safety assessment before regulators' approval[16, 32]. Previous studies reported the clinical safety and efficacy of β-glucan polysaccharide from oat and yeast[19, 33], while all published studies of β-glucan oligosaccharide are *in vitro* research[34, 35]. To our knowledge, this is the first human study to investigate the safety of both β-glucan polysaccharide and β-glucan oligosaccharide from the fungus. This dose-escalation study reports the MTD of β-glucan polysaccharide and β-glucan oligosaccharide from *O. dipterigena* BCC 2073 at the dose of 2,000 mg per day for a single intake and two weeks of repeated intake. This dose can be used further to study the long-term safety of repeated dose consumption.

Compared to the maximum limit of yeast β-glucan in food supplements defined by EFSA at 375 mg/ day [9], the maximum tolerated doses of β-glucan from *O. dipterigena* BCC 2073 are higher. This discrepancy may be due to distinct levels of research studies (animal studies for the yeast and our human study for the fungal one). Furthermore, the difference in structure between yeast and fungal glucan may also contribute. Though both are β-1,3/1,6 glucan, which contains 1,3 glycosidic linkages at the backbone and branching at 1,6, yeast-derived β-glucans have long β-(1, 6)-side branches while fungal β-glucan has short β-(1, 6)-linked branches [36-37]. Previous studies showed that smaller size can improve solubility, reduce viscosity, increase antioxidant activity, and immune activation effect[36-37]. Future studies are warranted to compare the safety and efficacy of yeast and fungal β-1,3/1,6 glucan. Throughout the supplementation with β-glucan, both the SAD phase and MAD phase, subjects with diarrhea and gastrointestinal discomfort were presented in the range from 8-16% in β-glucan polysaccharide group and 7-21% in β-glucan oligosaccharide group. However, no dose-dependent relationship was found with the increasing β-glucan intake and the symptoms are tolerable with no medical treatment required. β-glucan is a soluble fiber not digestible by the body; thus, it could alter bowel movement and defecation[38]. Previous studies show that β-glucan could affect intestinal permeability in the elderly with constipation and diarrhea[37]. A recent systemic review of clinical trials also suggests a small percentage of β-glucan's gastrointestinal side effects[19]. On the contrary, β-glucan was found beneficial in patients with irritable bowel syndrome[39].

In this study, supplementation of β-glucan oligosaccharide from the fungus *O. dipterigena* at a dose of 2 mg for 2 weeks significantly decreased blood glucose levels, while β-glucan polysaccharide also tends to reduce blood glucose levels, but its effect was not statistically significant. Previous studies of β-glucan polysaccharide from oats reported an effective dose of 3-6 g for 4-12 weeks for blood glucose control[40-41]. Therefore, the findings from the current study suggest that β-glucan isolated from the fungus may be more potent for blood glucose reduction than β-glucan isolated from oats. Future clinical studies are warranted to confirm such a hypothesis. Previous research proposed the mechanism of oat β-glucan for lowering blood glucose and lipid levels by increasing the viscosity of the digested food in the digestive tract and delaying nutrient absorption. The efficacy of β-glucan depends on the concentration, solubility, and the molecular weight of β-glucan[42]. This is consistent with our findings in this study that β-glucan oligosaccharides were more effective than polysaccharides in blood glucose control. Nevertheless, it is worth noting that the glucose-lowering effect observed in the current study did not show a dose-dependent manner. Since this study is conducted in healthy volunteers with normal blood glucose levels, the effect is statistically significant but still does not have clinical significance. Thus, we still cannot conclude the efficacy of 2 mg/ day as the effective dose. Future efficacy trials with various doses in people with pre-diabetes are recommended.

Besides blood glucose, a significant decrease in LDL level was found after intake of at least 1,500-2,000 mg of β-glucan polysaccharide for 2 weeks. Previous studies of β-glucan polysaccharide from oats reported an effective dose of at least 3 g/day for 12 weeks for cholesterol-lowering effect[20, 43]. Therefore, the findings from the current study suggest that β-glucan isolated from the fungus *O. dipterigena* BCC 2073 may be more potent for blood cholesterol control than β-glucan isolated from oats. Nevertheless, the two-week period in this study was too short to conclude. Future randomized control trials with a continuous intake of β-glucan from *O. dipterigena* BCC 2073 in hyperlipidemia subjects are encouraged.

It is worth noting that when β-glucan oligosaccharide and polysaccharide intake at doses of 1,000 mg or more, there was a trend of blood glucose and creatinine increase and a decrease in eGFR. However, the altered parameters were still within the normal ranges. The diet record data showed a significant increase in carbohydrate and overall energy intake with a reduction in dietary fiber intake during the study period

of dose 1,000-2,000 mg. Likewise, the protein intake also increases non-significantly. Kidney toxicity of β-glucan has never been reported. In contrast, oat β-glucan were shown to inhibit the progression of kidney disease and promote better kidney function in patients with chronic kidney disease stages 3-4[44]. Taken altogether, the observed increase in blood glucose and altered kidney markers in our current study is likely from the consumption behavior of regular food rather than a side effect of β-glucan.

The strength of this work includes the step-wise dose-escalation which allows us to monitor the participants' adverse events periodically, and the good compliance of all participants (minimal drop-out). The limitations of this study were the short duration of repeated intake (14 days) in the MAD study and the lack of a placebo control group to compare. A future randomized placebo control trial for repeated doses of β-glucan polysaccharide and β-glucan oligosaccharide capsules taken orally for a longer duration is warranted. The current study suggests that future long-term clinical trials should monitor adverse events, especially gastro-intestinal symptoms such as constipation and diarrhea. The placebo group is required to compare the adverse events and the changes in blood sugar, lipid profile, and kidney function. Diet records for monitoring carbohydrate, energy, and protein intakes are recommended.

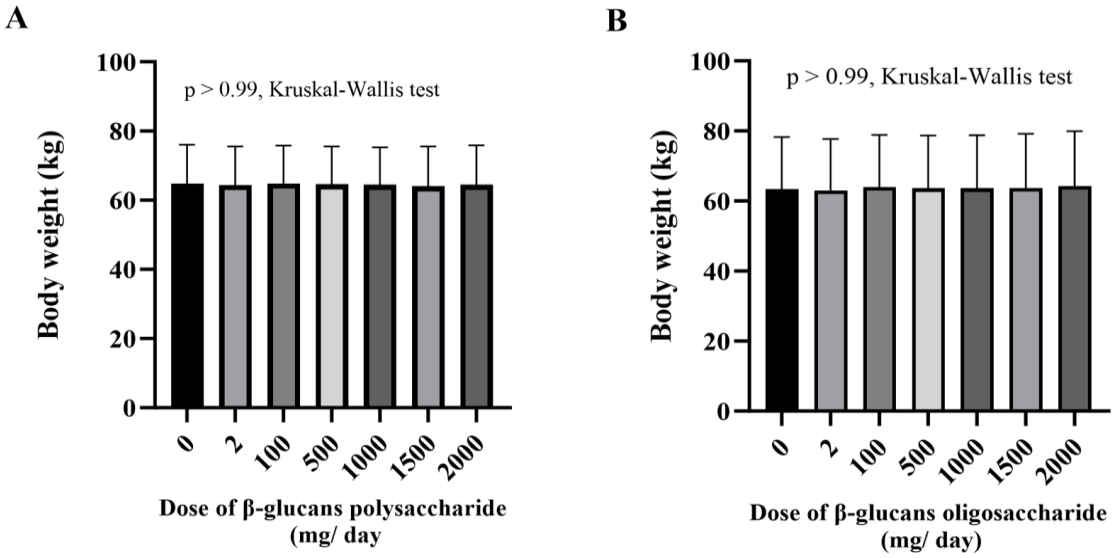


Figure 6. Changes in body weight before and after intake of β-glucans polysaccharide (A) or β-glucans oligosaccharide in MAD phase. The bar graph shows the mean ± SD of body weight in all participants (n=11 per group) at baseline (0), and after receiving 100, 500, 1,000, 1,500, and 2,000 mg of β-glucans polysaccharide or oligosaccharide once a day for two weeks each. The p-values were obtained from the Kruskal-Wallis test.

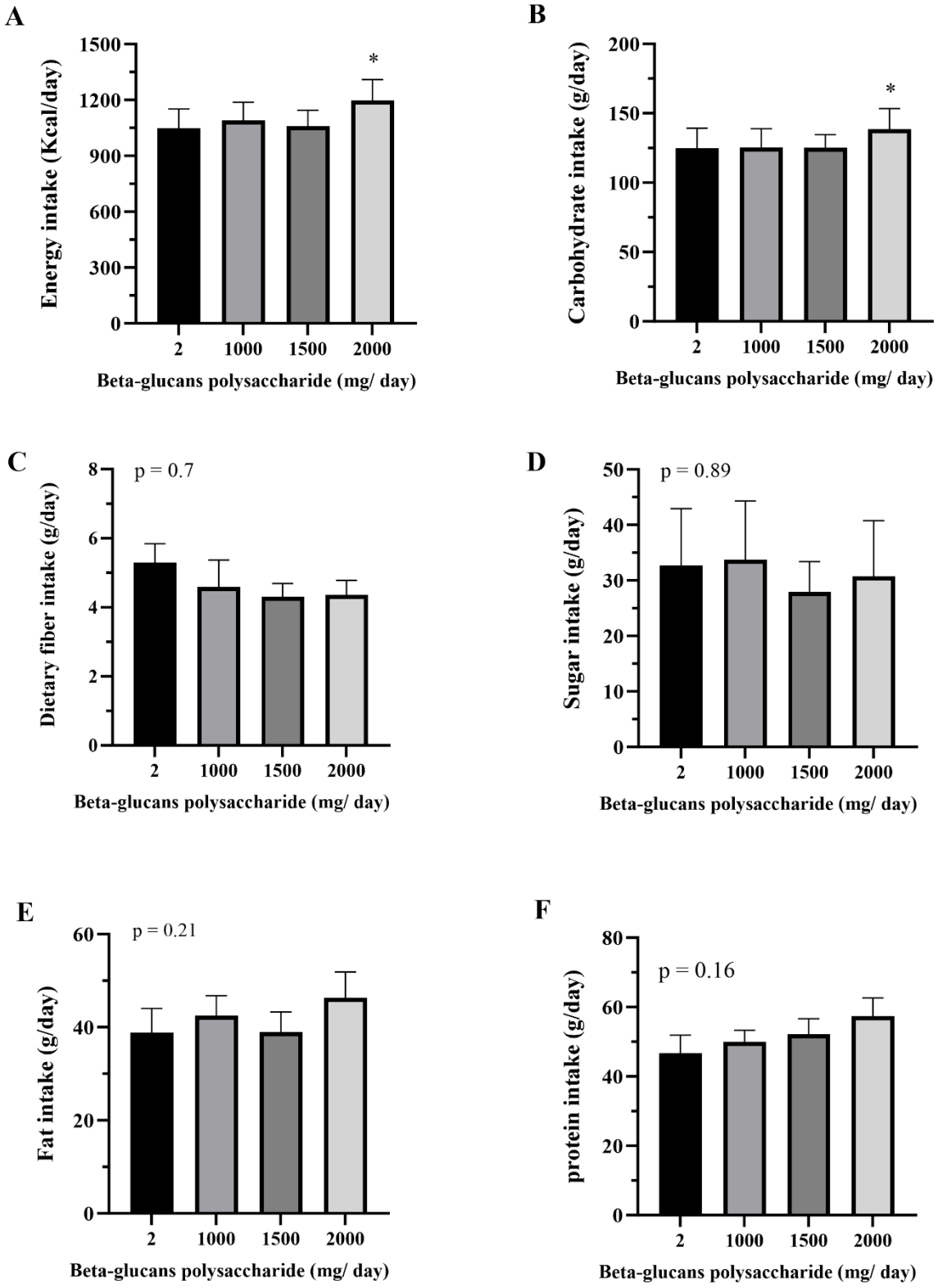


Figure 7. The nutrient intakes of participants during the supplementation of 2-2,000 mg of β-glucans polysaccharides in MAD phase. The bar graphs show mean ± SD. The p-values are derived from the Friedman test followed by Dunnett's multiple comparison test. * $p < 0.05$ compared to those of other doses.

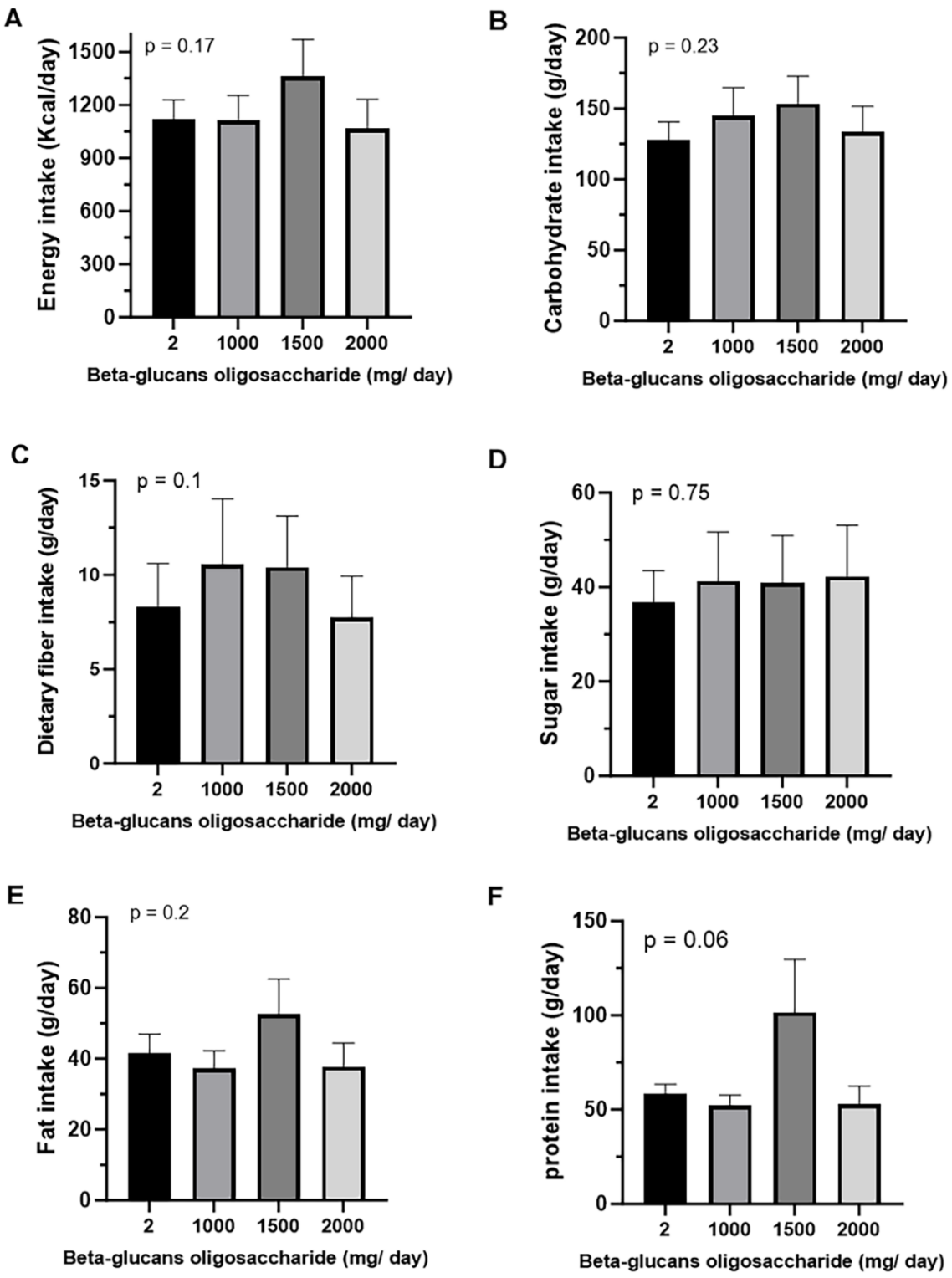


Figure 8. The nutrient intakes of participants during the supplementation of 2-2,000 mg β-glucans oligosaccharides in MAD phase. The bar graphs show mean ± SD. The p-values are derived from the Friedman test.

Conclusion

This study demonstrated that the supplementation of β-glucan polysaccharide and β-glucan oligosaccharide up to 2,000 mg once daily for two weeks is safe in healthy subjects. A future randomized, placebo-controlled trial is warranted to evaluate the long-term safety.

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Conflict of Interest

D.T. has received a research grant for this work from Asia Star Trade, Co., Ltd.., Thailand. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results. No competing financial interest exists for other authors. The authors have full control of all primary data.

Authorship

Funding acquisition and supervision, D.T.; Conceptualization and methodology, N.M., N.R., W.P., and D.T; Investigation, N.M., N.R., and D.T; Data curation, N.M., N.R.; Formal analysis, N.M., and D.T.; Writing-original draft, N.M. and N.R.; Writing-review and editing, D.T.; All authors have read and agreed to the published version of the manuscript.

Data availability statement

All data collected in this project have been described in this work. Since the participants only gave consent to report the data summary, no individual data can be shared publicly.

Compliance with ethical standards

Research involving Human Participants and/or Animals: The protocol of this study (MU-CIRB 2022/289.3110) was approved by the Mahidol University Central Institutional Review Board (MU-CIRB), approval number COA. No. MU-CIRB 2022/132.0812. Informed consent: written informed consents were obtained from all participants before data collection.

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