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Prebiotic capsules containing anthocyanin, inulin and rice bran extracts increased plasma ascorbate of overweight or obese subjects

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KEYWORDS
Antioxidant;
Dietary fiber;
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Anthropometry.

ABSTRACT
Prebiotic foods have an important role in health well-being, especially for treating overweight or obesity by promoting gut microbiota to improve antioxidant activity and fat metabolism. We aimed to investigate the effect of prebiotic capsules containing anthocyanin, inulin and rice bran extract on antioxidant, leptin and insulin resistance, and fat metabolism of overweight or obese subjects. The research was a randomized double-blinded controlled trial. Sixty overweight or obese subjects were randomly allocated into two groups; 1) 350 mg/prebiotic capsule (prebiotic group) or 2) 350 mg/maltodextrin (placebo group), 3 capsules after each meal and before going to bed (4.2 g.day-1) for 30 days. Before and after 30 days of supplementation, anthropometry and body composition were measured. Blood samples were collected to analyze ascorbate, glucose, lipid profiles, leptin and insulin concentrations. An expired gas was collected for 5 min to determine substrate utilization. The results showed prebiotic capsules increased plasma ascorbate concentration (placebo group: before 34.6 ± 3.33 and after 45.2 ± 4.48 µmol/L; treatment group: before 35.7 ± 4.43 and after 62.3 ± 6.47 µmol/L, 95% CI 16.1(4.27-27.9), *p*-value < 0.05). Comparing within group, hip circumference, lipid profiles, and leptin and insulin concentrations were significantly decreased with prebiotic supplementation comparing to the baseline data (*p*-value < 0.05). However, there were no significant changes from baseline in fat and carbohydrate oxidation rates, plasma glucose, liver and kidney functions within and between groups (*p*-value < 0.05). In conclusion, supplementation of prebiotic capsule containing 1.89 g anthocyanin, 1.89 g inulin and 0.42 g rice bran per day for 30 days increased plasma ascorbate concentration in overweight or obesity subjects. Daily supplementation of 4.2 g prebiotics for 30 days did not cause liver and kidney dysfunctions. However, either higher dose or longer duration of the supplementation is warranted to show more variables those are significant and to confirm the safety.

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Introduction

Worldwide prevalence of overweight and obesity has doubled increment since 1980 to nearly a third of the world's population[1]. According to the Asian-Pacific cutoff points, classification of overweight by body mass index (BMI) is between 23 and 24.9 kg/m^2, and obesity is higher than 25 kg/m^2[2]. BMI is demonstrated to be associated with oxidative stress, in consequence of impaired antioxidant status such as vitamin C[3], abnormal lipid metabolism[4] and gut microbiota[5]. Amount of short-chain fatty acids (SCFA) (such as acetate and l-lactate) was increased by fermentation of carbohydrates by microbiota[6]. The SCFA is used as metabolic substrates by the host cell to increase the energy determined by fatty acid oxidation. Besides, the microbiota dysfunction contributes to leptin[7] and insulin resistance[8]. Accordingly, overweight and obesity increase risks of many life-threatening diseases such as diabetes mellitus type 2[9]. Prebiotics are food components that induce the growth or activity of beneficial microbiota. Thus, prebiotic food is an alternative choice being developed for treating overweight and obesity[10].

Prebiotics used in this study are anthocyanin from black rice as an antioxidant and inulin from Jerusalem Artichoke and rice bran from rice mill as indigestible dietary fiber. The antioxidant activity has the potential to improve gut microbiota, which contributes to improved metabolic health. Many studies showed that the supplementation of anthocyanin extracted from black rice bran 1.8 g/day for 8 weeks displays very high antioxidant and anti-inflammatory activities resulting in a decrease in blood lipid concentrations and protecting the arteries[11-13]. Anthocyanin 2.5 g/day for 8 weeks also decreased abdominal fat, waist and hip circumferences of the people with overweight or obese[14,15].

The indigestible dietary fiber including inulin from Jerusalem artichoke and rice bran from Glutinous Rice RD6 dissolved in water increases viscosity leading to the delay of gastric emptying time. This dietary fiber can be digested and fermented by bacteria in the large intestine resulting in an increase of short-chain fatty acids (SCFAs)[6]. Dietary fiber also helps to increase the utilization of glucose and fat resulting in weight loss in overweight or obese people[16]. In addition, a previous study reported that a combination of inulin (4 g/day) and anthocyanin (163 mg/day) for 4 weeks can stimulate the growth of microorganisms in the digestive tract and increase satiety hormone and improves blood glucose tolerance[17]. Moreover, there are no previous studies exploring the effects of capsule containing all three prebiotics on various variables and on liver and kidney functions.

Taken together, we therefore aimed to investigate the effects of prebiotic capsule containing 1.89 g anthocyanin, 1.89 g inulin and 0.42 g rice bran extract per day for 30 days primarily on antioxidant, and subsequently on leptin and insulin resistance, and fat metabolism of overweight or obese subjects. We used plasma ascorbate to indicate antioxidant because it plays role in increased fat oxidation[18] which has been shown to improve insulin sensitivity[19]. Besides, it was demonstrated to improve lipid profile including cholesterol[20,21]. We hypothesized that the supplementation of the prebiotic capsule would improve the above variables for overweight or obese subjects.

Materials and methods

Subjects

All subjects signed an informed consent after being clarified by both verbal and writing information. Our research has been approved by the Khon Kaen University Ethics Committee on Human Research in accordance with the declaration of Helsinki (HE601277) and was registered in Thai Clinical Trials (TCTR20180317002) on March 14, 2018. The subjects were recruited if they met these criteria: males and females, aged 20-50 years with overweight (BMI between 23 and 24.9 kg/m^2), or obesity (BMI equal to or more than 24.9 kg/m^2)[2], no underlying diseases such as diabetes mellitus, cardiovascular, neuromuscular disorders, liver and kidney diseases. All subjects received the preliminary test of physical characteristics and anthropometry examinations including the height, mass, body

mass index (BMI), waist and hip circumferences, waist/hip ratio and electrocardiography (ECG). Their whole-body fat and muscle mass were assessed using Dual-Energy X-Ray Absorptiometry (DXA). Blood sample was collected to analyze anemia by measuring complete blood count, liver function by measuring serum glutamic-pyruvic transaminase (SGPT), kidney function by measuring blood urea nitrogen (BUN) and creatinine (Cr). Also, blood glucose, insulin and lipid profiles were analyzed. Moreover, the dietary intake and physical activity were recorded 3 days a week; 2 days on weekdays (Mon-Fri) and one day on weekends (Saturday or Sunday). The dietary data were reported to calculate total energy intake and the percent of three major nutrients (protein, carbohydrate, and fat) per day using the Inmucal-nutrients® version 3 software (Institute of Nutrition, Mahidol University, Thailand).

Study design

This research is a randomized, double-blinded, placebo, parallel controlled trial. Sixty subjects (51 females and 9 males) with overweight or obesity were divided into two groups; 1) 350 mg/ prebiotic capsule, 3 capsules 4 times a day for 30 days (4.2 $g.day^{-1}$) (prebiotic group) or 2) 350 mg/ maltodextrin (placebo group).

The sixty subjects including 15% dropout in this study were obtained based on the study of Parnell and Reimer, 2009[22]. The authors reported a statistically significant decrease in body weight 1.03 ± 0.4 kg due to the effects of oligofructose inulin. Therefore, this reported using researcher respect to the volunteers may have body weight decrease as 0.7 ± 0.43 kg. According to statistical calculation by program G' power 3.0, alpha concentration as 0.05 and power at 80% sample size of this study was 26 subjects and dropout 4 subjects in each group.

Protocol

After subjects passed the screening, they visited the laboratory for 3 occasions; visit 1, for pre-test and received the first half pack of the supplement capsules, visit 2, for blood sampling for liver and kidney functions and received the second half pack of the supplement capsules, and visit 3, for the post-test. All subjects did not change their daily dietary intake and physical activity throughout the experiment. Moreover, this study compliance was followed up by phone once a week. Before and after the supplementation. Five ml of blood samples were collected and divided into 5 of 1 ml tubes to measure glucose (sodium fluoride tube), ascorbic acid (lithium heparin tube wrapped with aluminum foil), insulin (SST gel tube), lipid profiles and leptin (EDTA tube). Before and after the supplementation, expired gas was collected for 5 min to determine oxygen uptake ($\dot{V}O_2$) (L/min) and carbon dioxide production ($\dot{V}CO_2$) (L/min) using a gas analyzer (Jaeger Oxycon Mobile; Jaeger, Germany). Carbohydrate and fat utilization were calculated based on $\dot{V}O_2$ and $\dot{V}CO_2$.

Preparation of prebiotic capsule containing anthocyanin, inulin and rice bran

Prebiotic capsule containing anthocyanin, inulin and rice bran extract (45:45:10) and placebo capsule containing maltodextrin were prepared under good manufacturing practices (GMP) in the Faculty of Food Technology, Khon Kean University, Thailand. Each prebiotic capsule contains 1.89 g anthocyanin, 1.89 g inulin, and 0.42 g rice bran extract. Placebo capsule contained 350 mg maltodextrin powder with the same color, size, and odor of the prebiotic. Both prebiotic extracts and placebo were in green color capsules.

The followings were the methods of the anthocyanin, inulin, and rice bran extracts and placebo production.

- Anthocyanin extract: black rice was boiled with hot water 85 ºC for 30 minutes and then the waste was separated. The liquid part was mixed to 20% maltodextrin and anthocyanin powder was produced by spray dryer machine at 160 ºC.
- Inulin extract: fresh Jerusalem artichoke was washed with clean water. Then it was cut into thin sheets and dried in a hot air oven at 60ºC for 12-14 hours until it had a moisture content of 9-10%. Then, it was boiled in hot water at 85-90 ºC for 30 minutes, after that the liquid was removed and mixed with 5% maltodextrin. The inulin powder was produced by spray dryer machine at 160 ºC.

- Rice bran (soluble dietary fiber) extract: glutinous rice RD6 was crushed and boiled in hot water with 95ºC for 20 minutes. After that, the liquid was removed by filtering. The dietary fiber powder was produced by spray dryer machine at 160 ºC.
- The prebiotic and placebo capsules were kept in zip lock bag labeled with expiration dates and the method required for oral ingestion. Both capsules were stored at 4 ºC in a refrigerator for maintaining quality of active ingredients.

All subjects took 350 mg/capsule 4 times a day (4.2 g/day) for 30 days. Contaminant level was measured for safety by the Central Laboratory, Thailand (Co., Ltd). The prebiotic and placebo capsules were kept in zip lock bags labeled with expiration dates and the method of how to ingest orally. All capsules were stored at 4 ºC in a refrigerator to maintain the quality of active ingredients until we use.

Outcome measurements

Plasma ascorbate

One mL of whole blood was centrifuged 3,000 x g at 4 ºC for 10 min. The upper plasma layer was separated and added with 1 mol/L $HClO_4$ to precipitate protein. Then, the tubes were centrifuged and wrapped with aluminum foil to be protected from sunlight. The upper solution was stored at -80ºC until analyzed. Plasma ascorbate concentration was analyzed by Zhang's assay[23]. In this assay method, Fe (III) was deoxidized to Fe (II) by ascorbate at pH 4.0 and Fe (II) reacted with potassium ferricyanide to form a blue product, soluble prussian blue [KFe^{III} [$Fe^{II}(CN)_6$]. The absorbance was measured using a spectrophotometer at 735 nm. The amount of ascorbate was calculated using the standard curve.

Plasma leptin

Plasma leptin concentration was measured using an enzyme immunometric assay (Human Leptin Enzyme Immunoassay Kit of SPI-Bio®, France) as a double-antibody sandwich technique. The wells of plate supplied in the kit were coated with a polyclonal antibody specific of human leptin. The plasma 100 µl and EIA buffer 100 µl was put on 96 wells. The plate was incubated in the dark at 20°C for 10 min to avoid being exposed to direct sunlight. Aluminum foil was recommended to be used to cover the plate. The activity developing color was stopped by adding 100 µL of stop solution and read the absorbance by ELISA plate reader at 450 nm within 5 min after addition of the stop solution.

Other biochemical analyses

Before and after the prebiotic supplementation, after 12 hours of overnight fasting blood, samples 3 mL were collected from the antecubital vein. Blood glucose concentrations were measured using the glucose oxidase method (YSI 2300 STAT Plus™, USA, which was auto calibrated in every five sample test) immediately after blood collection. Serum insulin concentration was analyzed using a radio-immunoassay kit (ImmuChem™ Coated Tube kit of MP Biomedicals®, USA). In addition, homeostatic model assessment for insulin resistance (HOMA-IR) was calculated using the equation of {HOMA-IR formula = Fasting glucose (mmol/L) x Fasting insulin (mIU/L)/22.5}. From 2 mL blood sample in EDTA-tubes, 32 µL was used to analyze plasma lipid profile, creatinine, BUN and SGPT by Reflotron strips (Reflotron® Plus, Boehringer Mannheim, German

Substrate oxidation rate

Carbohydrate and fat oxidation rates were calculated based on $\dot{V}O_2$ (L/min) and $\dot{V}CO_2$ (L/min) using the Peronnet and Massicotte equation ignoring protein oxidation rate[24].

Statistical analysis

Normal distributions were tested with the Kolmogorov-Smirnov test. Statistical analyses were performed using SPSS 19 package software. To compare within group difference (before and after), paired *t* test was used. The difference between the grouped volunteers was analyzed using an analysis of covariance (ANCOVA). Statistically significant difference was taken at *p*-value < 0.05. Data are expressed as mean ± SD except stated elsewhere.

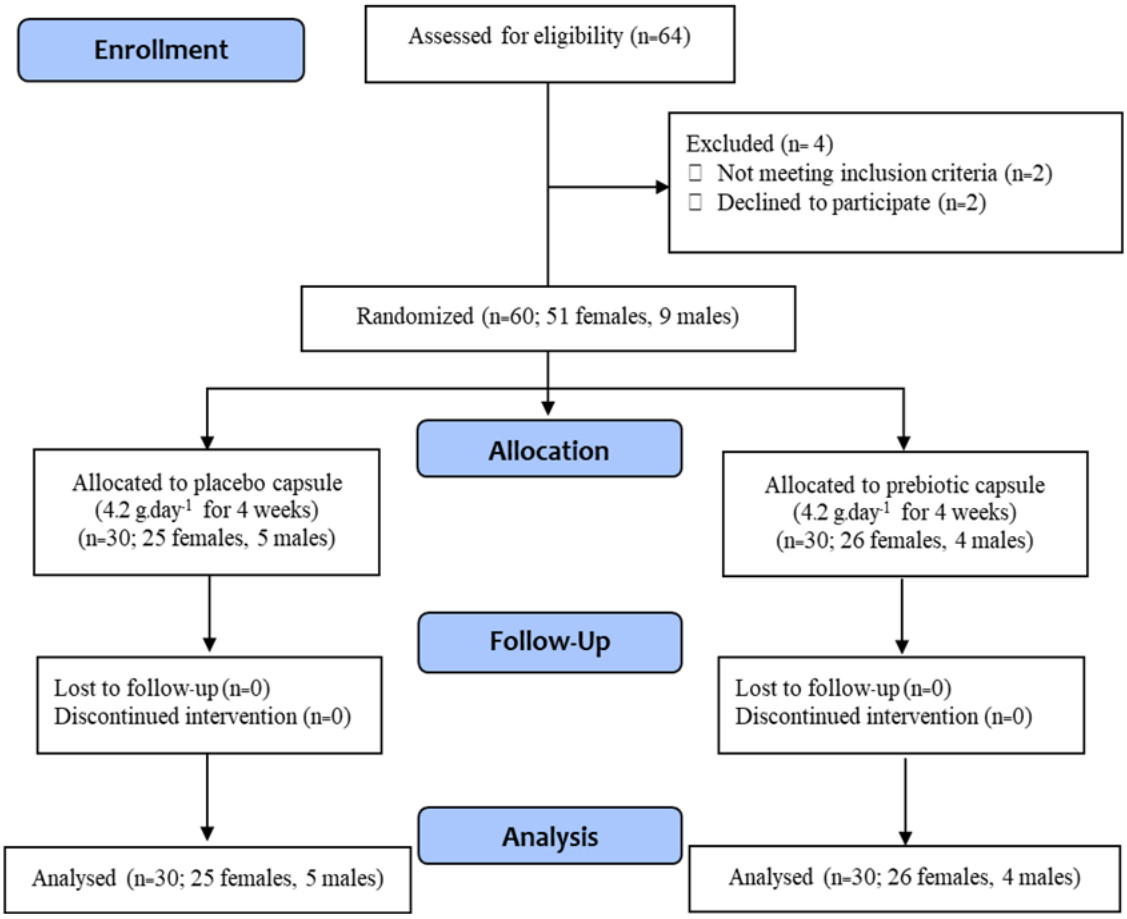

Figure 1 Flow chart of the trial protocol

Results

Originally this study had 64 subjects (54 females, 10 males). However, four subjects were excluded because they did not meet the inclusion criteria (n=2) and decided not to participate (n=2). Sixty subjects were separated into 2 groups with 30 persons each group, placebo group (25 females, 5 males) and prebiotic group (26 females, 4 males), whose data and samples completely collected with the study request in Figure 1.

Baseline physiological characteristics and energy intake and expenditure

The baseline physiological characteristics of all subjects were summarized in Supplementary Table 1. The characteristics of subjects including age, height, body mass, BMI, and blood pressure were not significantly different between placebo and treatment groups. Moreover, as shown in Supplementary Table 2, daily dietary components of energy intake, and energy expenditure did not differ between placebo and treatment groups (p-value > 0.05).

Anthropometry and body composition

After supplementation of placebo and prebiotic capsules, body mass, fat mass, lean mass, BMI, waist and hip circumferences, waist/hip circumference ratio were not significantly different (p-value > 0.05) before and after supplementation between both groups except hip circumference of the treatment group which was significantly (p-value < 0.05) lower than that of the baseline in Supplementary Table 3.

Plasma ascorbate

Plasma ascorbate concentrations after treatment was significantly higher in the prebiotic treatment group than those in the placebo group (p-value < 0.05). Both placebo and treatment groups showed an increase in ascorbic acid concentrations compared to that at the baseline (p-value < 0.05) in Table 1.

Plasma glucose, leptin, insulin, lipid profiles

While leptin and insulin concentrations of the placebo group increased after treatment, those of the prebiotic treatment group decreased from baseline after treatment (p-value < 0.05) in Table 1. Plasma leptin and insulin concentrations after supplementation did not significantly differ between the treatment and placebo groups.

Total cholesterol concentration was significantly decreased (p-value < 0.05) after prebiotics supplementation, whereas there were no changes in placebo group (Table 1). Insulin resistance was significantly increased in control group (p-value < 0.05), whereas it did not change after prebiotics supplementation. In addition, glucose, triglyceride, LDL, HDL, TC/HDL ratio, and LDL/HDL ratio were not significantly different within and between groups. Insulin, HDL, and leptin are tended to be lower than the control group in Table 1.

Substrate utilization

Carbohydrate and fat oxidation rates, as well as, the percentage of carbohydrate and fat contribution to total energy expenditure were not significantly different within and between groups in Table 2.

Liver and kidney functions

There were no significant changes in SGPT, BUN and creatinine concentrations before and after supplementation in both groups (Table 3). Also, SGPT, BUN, and creatinine concentrations were within normal range. These values confirmed the safety of the prebiotics capsule on liver and kidney functions.

Table 1 Blood variables of subjects in both groups

Variables	Placebo group		Prebiotic group		Mean difference	95% CI		*p*-value
	Before	After	Before	After		Lower	Upper	
Plasma glucose (mg/dL)	92.50 ± 3.90	94.20 ± 3.42	101.70 ± 4.88	98.00 ± 3.67	-2.89	-12.91	7.13	0.57
Insulin (µIU/mL)	5.29 ± 0.82	6.70 ± 0.86*	7.82 ± 1.77	7.07 ± 1.17*	1.81	-0.05	3.66	0.06
Insulin resistance (HOMA-IR)	1.21 ± 0.19	1.61 ± 0.10*	1.55 ± 0.20	1.32 ± 0.20	0.33	-0.11	0.77	0.14
TC (mg/dL)	202.20 ± 6.18	190.50 ± 8.07	212.10 ± 8.43	197.00 ± 8.03*	1.33	-14.71	17.36	0.87
TG (mg/dL)	116.50 ± 8.41	106.30 ± 7.68	118.20 ± 10.41	107.60 ± 7.88	-0.66	-20.29	18.96	0.95
LDL (mg/dL)	123.90 ± 5.50	112.50 ± 8.35	126.50 ± 7.37	119.70 ± 6.30	-1.83	-17.02	13.37	0.81
HDL (mg/dL)	54.90 ± 2.17	56.80 ± 4.06	57.40 ± 3.03	55.70 ± 3.32	6.82	-1.03	14.66	0.09
TC/HDL ratio	3.83 ± 0.18	3.76 ± 0.31	3.72 ± 0.22	3.67 ± 0.24	0.17	-1.22	1.56	0.81
LDL/HDL ratio	2.42 ± 0.14	2.29 ± 0.22	2.20 ± 0.18	2.17 ± 0.14	0.26	-1.08	1.59	0.70
Plasma ascorbate (µmol/L)	34.60 ± 3.33	45.20 ± 4.48*	35.70 ± 4.43	62.30 ± 6.47*#	16.08	4.27	27.88	0.01*
Plasma leptin (mg/mL)	4.40 ± 0.36	4.76 ± 0.37*	4.84 ± 0.32	4.66 ± 0.31*	0.47	-0.05	0.99	0.074

Note: Data are expressed as mean ± SE; n = 30 in each group (placebo group: 25 females, 5 males and prebiotic group: 26 females, 4 males); TC, total cholesterol; TG, triglyceride; LDL, low-density lipoprotein; HDL, high density lipoprotein.

*Significantly different from before supplementation (*p*-value < 0.05).

#Significantly different from placebo group at the same condition (*p*-value < 0.05).

Table 2 Substrate utilization of subjects in both groups

Variables	Placebo group		Prebiotic group		Mean difference	95% CI		*p*-value
	Before	After	Before	After		Lower	Upper	
CHO oxidation rate (g/min)	0.12 ± 0.03	0.14 ± 0.02	0.12 ± 0.03	0.13 ± 0.02	0.01	-0.05	0.07	0.74
Fat oxidation rate (g/min)	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.01	-0.01	0.03	0.28
CHO oxidation rate (mg/kg.BM/min)	3.18 ± 0.77	3.61 ± 0.65	3.33 ± 0.63	3.49 ± 0.43	0.12	-1.51	1.76	0.88
Fat oxidation rate (mg/kg.BM/min)	2.00 ± 0.28	1.80 ± 0.20	1.86 ± 0.23	1.59 ± 0.20	0.03	-0.58	0.63	0.93
% CHO contribution to total energy expenditure	42.50 ± 8.87	45.20 ± 7.31	42.00 ± 8.82	50.70 ± 5.46	-4.02	-20.78	12.74	0.63
% Fat contribution to total energy expenditure	57.50 ± 8.87	54.90 ± 7.31	58.00 ± 8.82	49.30 ± 5.46	4.02	-12.74	20.78	0.63

Note: Data are expressed as mean ± SE; n = 30 in each group (placebo group: 25 females, 5 males and prebiotic group: 26 females, 4 males); CHO, carbohydrate.

Table 3 Blood variables determining liver and kidney functions of subjects in both groups

Variables	Placebo group		Prebiotic group		Mean difference	95% CI		*p*-value
	Before	After	Before	After		Lower	Upper	
SGPT (μ/L)	17.40 ± 1.65	17.90 ± 2.56	17.20 ± 2.18	17.20 ± 1.38	4.67	-2.44	11.78	0.19
BUN (mg/dL)	12.70 ± 0.44	13.30 ± 0.44	12.60 ± 0.51	12.80 ± 0.45	0.51	-0.63	1.64	0.38
Creatinine (mg/dL)	0.84 ± 0.03	0.78 ± 0.04	0.76 ± 0.04	0.78 ± 0.03	-0.03	-0.11	0.05	0.50

Note: Data are expressed as mean ± SE; n = 30 in each group (placebo group: 25 females, 5 males and prebiotic group: 26 females, 4 males); SGPT, serum glutamic-pyruvic transaminase; BUN, blood urea nitrogen.

Discussion

The present results show that supplementation of 4.2 g/day prebiotic capsule containing anthocyanin, inulin, and rice bran extract for 30 days increased fasting plasma ascorbate concentration. Also, prebiotic supplementation could decrease plasma triglyceride, insulin, and leptin concentrations and hip circumference. However, both prebiotic treated and placebo treated groups did not show any changes of substrate oxidation rates, blood glucose and insulin resistance. Prebiotic supplementation for 30 days did not affect liver and kidney functions.

We hypothesized that supplementation of the prebiotic capsule containing anthocyanin, inulin and rice bran extract would improve primarily on antioxidant, and subsequently on leptin and insulin resistance, and fat metabolism of overweight or obese subjects. The results partially supported our hypothesis since prebiotic supplementation significantly increased plasma ascorbate concentration, which has antioxidant activity[25]. For now, there have been no previous reports of the influence of any one of, or combination of, the three extracts on plasma ascorbate of overweight or obese subjects. Only

one previous work on those prebiotics was the research in healthy subjects[26]. Their results supported our findings although they used less anthocyanin amount than ours. They reported that 400 mg/50 kg body weight of anthocyanin-rich food increased plasma ascorbate concentration in healthy subjects with 50-65 kg body weight[26]. Anthocyanin also increased activity of another antioxidant such as superoxide dismutase (SOD). Intake of 750 mL bolus of anthocyanin-rich fruit juice containing 205.5 mg anthocyanin daily for 9 weeks significantly increased SOD activity[27]. On the other hand, Ellinger et al. (2012) did not find the antioxidant activity following 400 mL of fruit juice (27.9 mg anthocyanin) consisted of açai, camu-camu and blackberry in healthy nonsmokers, and this may be due to the insufficient dose of anthocyanin in the latter[28]. Another prebiotic, inulin, also has antioxidant activity in vitro and in vivo in animal and human studies. In vitro experiment showed that a DPPH radical scavenging activity of inulin at doses of 0.25 to 10 mg/mL increased linearly ($R2$ = 0.985, p-value<0.05)[29]. In animal experiment, inulin supplementation at the doses of 10, 15 and 20 g/kg increased antioxidant enzyme activities of SOD, catalase, and glutathione peroxidase and the total antioxidant capacity[29]. Likewise, supplementation of 10 g/day oligofructose-enriched inulin has antioxidant effect on women with type 2 diabetes[30]. In contrast, Hasan et al. (2018) could not find the antioxidant effect of intake of 30 g flavored-rice bran powder and 220 ml rice bran oil for 14 days in obese subjects[31], and this may be due to insufficient supplementation time. Further researches investigating the effects of the capsule in the present study on those antioxidants are needed.

In the present study, we found the significant improvement on total cholesterol, leptin and insulin concentrations, and hip circumference after prebiotic supplementation in prebiotic group and tended to be more improved than control group. There have not been any studies reporting the effects of the combination of three extracts on total cholesterol, insulin and leptin hormones and hip circumference. A review literature[32] reported that anthocyanin could reduce total cholesterol concentration. Studies on inulin at the dose of 7 g/day for 4 weeks to obese and 10 g/day for 8 weeks to diabetes type 2 subjects decreased total cholesterol[30,33]. A previous study reported also that plasma total cholesterol concentration of 12 healthy male subjects decreased after taking 50 g of cereals containing 18% inulin[34]. In this study, in agreement with Van der Beek et al.[35], plasma insulin concentration was reduced in the prebiotic supplemented group. Van der Beek et al.[35] investigated the effects of 24 g inulin in high-fat milk shake supplementation and found the decrease of plasma insulin concentration 3 hr after the consumption during 7 hr follow-up. Furthermore, 10 g/day inulin supplementation decreased plasma insulin concentration at 4 weeks in patients with type I and II diabetes mellitus[34,36]. No publication is available on the effect of anthocyanin, inulin, or rice bran on leptin concentration of people with overweight/obesity. In addition, anthocyanin-rich black soybean extracts (2.5 g/day) for 8 weeks was shown to decrease hip circumference but was not significantly different from control group in people with overweight/obese[14]. Administration of inulin 9 g/day for 18 weeks to obese dyslipidemia subjects could decrease hip circumference although it statistically was not different from control group[37]. No reference is available on the effect of consumption of rice bran on hip circumference.

In this study, other outcomes i.e. substrate oxidation rates, blood glucose, and leptin and insulin resistance were not affected by the supplementation of combined prebiotics. These results are inconsistent with previous studies. Administration of 11 g/day anthocyanin for 4 weeks was reported to improve plasma glucose concentration[38]. Similarly, rice bran at 10 g/day for 8 weeks also reduced hyperglycaemia of diabetes mellitus Types I and II patients[39]. The dose and duration of the prebiotics supplementation in this study may not be enough to cause the significant effects.

Theoretically, prebiotic supplements might augment their fermentation with gut microbiota resulting in the increase of SCFAs. The SCFAs can easily enter mitochondria leading to increase fat oxidation and providing energy for the cell. Inulin at doses of 23.5 and 24 g increased fat oxidation. rate of the people with overweight/obesity[35,40]. The increased fat oxidation rate resulted in the reduction of circulating FFA[35], lipid profile[11]. Likewise, 30 g inulin for 18 weeks to prediabetes patients reduced hepatic and muscle fat[41], and 1.8 g rice bran for 8 weeks to uncontrolled overweight/obesity could reduce body fat[11] and body mass[41]. The other mechanism of the effects of prebiotics is to improve *Lactobacillus* production which in turn controlling the TNF-α and leptin gene expression. Anthocyanin 200 mg/day consumption for 4 weeks significantly reduced blood leptin concentration[7], supporting our results of decreased leptin concentration in the prebiotic treated group. Besides, the previous study also showed anthocyanin could decrease body weight and BMI[7]. The body fat[11] and weight reduction[42] may contribute to decreased insulin resistance. The improved insulin resistance results in decreased blood glucose as shown in the previous studies of inulin supplementation[41] and HbA1c in healthy were significantly reduced after inulin supplementation[30].

In this study, prebiotic treatment of 4.2 g/day capsule for 30 days did not cause any damage of liver and kidney functions. Supportive of this, Mahadita et al. (2016) reported that anthocyanin 11 g/day for 4 weeks were safe on liver and kidney function measured by SGPT, SGOT, BUN, and Cr[38]. Besides, inulin 10 g/day for 2 months significantly decrease SGOT and SGPT but did not change Cr[43]. Moreover, 30 g/day rice bran for 6 months decreased SGOT and SGPT[44]. The doses of extracts and duration of the supplementation that we used in this study are lower[38,43,44] and shorter[43,44] than those in previous studies. Therefore, no damages in the liver and kidney were found in our participants.

There are several limitations of this study. Firstly, there is a lack of data on oxidants and other antioxidants of both enzymatic and non-enzymatic nature. The influence of the prebiotics used in this study on oxidants and other antioxidants should be investigated further. Another important missing investigation is the type and amount of microbiota and concentration of SCFA. The results may provide knowledge of the molecular mechanism explaining the effect of these prebiotics. The other limitation is the gender imbalance since a ratio of females vs males is 1:5 in control group and 1:6 in this study. A very recent study suggested that gender had an effect on the impact of dietary fiber on the gut microbiome[45]. Thus, the results of this study cannot be applied in male population. Besides, the future study should be taken on a higher dose and a longer duration because some data might be affected by higher doses and prolonged treatment. Moreover, the effects of the prebiotic capsule on proposed variables should be investigated in the population with high oxidative stress such as diabetes mellitus. This will yield beneficial effect of the prebiotic supplementation to more population, such as diabetes mellitus.

Conclusion

Our findings suggest that 30-day supplementation of prebiotic capsule containing 1.89 g anthocyanin, 1.89 g inulin and 0.42 g rice bran per day increased plasma ascorbate which is non-enzymatic antioxidant in individuals with overweight or obesity. However, it did not influence other outcomes. Either higher dose or longer duration of the supplementation may result in the significant effects. The prebiotics used in this study did not cause any serious toxic effects on liver and kidney.

Clinical implications

- Anthocyanin, inulin, and rice bran supplement increased plasma ascorbate concentration in overweight or obesity subjects.
- In prebiotic group, hip circumference, lipid profiles, and leptin and insulin concentrations were decreased.
- This 30-day prebiotic supplementation did not cause liver and kidney damage.

Conflicts of interest

The authors declare no conflict of interest.

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Supplementary data

Supplementary table 1 Baseline characteristics of subjects

Variables	Control group	Prebiotic group	*p*-value
Gender (female/male)	25/5	26/4	0.73
Age (yr)	38.00 ± 9.48	38.00 ± 8.83	0.99
Height (m)	1.58 ± 0.09	1.58 ± 0.08	0.54
Body mass (kg)	73.70 ± 14.99	72.50 ± 10.39	0.71
BMI (kg/m^2)	29.50 ± 4.80	28.90 ± 3.30	0.97
Systolic (mmHg)	120.00 ± 13.58	123.00 ± 14.00	0.47
Diastolic (mmHg)	77.00 ± 12.62	79.00 ± 13.24	0.49
Heart rate (/min)	76.00 ± 8.28	78.00 ± 11.11	0.90

Note: Data are expressed as mean ± SD; n = 30 in each group; control group, prebiotic group; BMI, body mass index.

Supplementary table 2 Physical activity and dietary assessment of subjects

Variables	Control group	Prebiotic group	*p*-value
Protein (g)	87.30 ± 3.83	84.30 ± 4.52	0.62
Carbohydrate (g)	219.30 ± 9.58	232.10 ± 9.14	0.34
Fat (g)	67.60 ± 4.20	62.80 ± 3.02	0.36
Potassium (mg)	1456.50 ± 126.96	1359.50 ± 119.92	0.59
Sodium (mg)	3064.80 ± 377.36	3100.40 ± 353.06	0.95
Vitamin A	224.70 ± 38.36	271.30 ± 44.19	0.45
Thiamine (mg)	1.00 ± 0.13	1.07 ± 0.13	0.74
Riboflavin (m)	1.16 ± 0.10	1.08 ± 0.09	0.53
Vitamin B6 (mg)	0.39 ± 0.06	0.38 ± 0.07	0.67
Vitamin B12 (mg)	0.91 ± 0.18	1.19 ± 0.47	0.68
Vitamin C (mg)	35.96 ± 6.99	35.06 ± 5.56	0.88
Vitamin E (mg)	0.81 ± 0.12	0.74 ± 0.18	0.22
Crude fiber (g)	0.60 ± 0.09	0.69 ± 0.14	0.89
Dietary fiber (g)	6.67 ± 0.74	6.37 ± 0.87	0.79
Energy intake (Kcal/day)	1836.70 ± 11.06	1838.60 ± 9.80	0.90
Energy expenditure (Kcal/day)	1692.30 ± 84.66	1694.10 ± 76.07	0.70

Note: Data are expressed as mean ± SD; n = 30 in each group; control group, prebiotic group.

Supplementary table 3 Anthropometric and body composition of subjects

Variables	Control Group		Prebiotic Group		Mean difference	95% CI		*p*-value
	Before	After	Before	After		Lower	Upper	
BMI (kg/m^2)	29.50 ± 0.88	29.60 ± 0.87	28.90 ± 0.60	28.30 ± 0.83	0.69	-0.67	2.06	0.31
Body mass (kg)	73.70 ± 2.74	73.90 ± 2.70	72.50 ± 1.90	70.90 ± 2.35	1.84	-1.80	5.48	0.32
Fat mass (kg)	29.50 ± 1.45	29.60 ± 1.46	29.80 ± 1.41	31.20 ± 2.22	0.41	-0.82	1.65	0.50
% Fat android	48.30 ± 0.74	48.40 ± 0.82	50.30 ± 1.13	50.40 ± 1.12	-0.19	-1.34	0.97	0.75
% Fat gynoid	47.20 ± 1.31	47.80 ± 1.40	48.60 ± 0.97	47.80 ± 0.94*	0.86	-0.19	1.90	0.10

Note: Data are expressed as mean ± SE; n = 30 in each group; control group (25 females and 5 males), prebiotic group (26 females and 4 males); BMI, body mass index.
*Significantly different from before supplementation (p-value < 0.05).