Original Article

A Randomized, Double Blind Clinical Study to Assess the Effects of a Gamma-oryzanol-enriched Rice Bran Oil on Lipid Profile in the Hypercholesterolemic Patients

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Background: Hypercholesterolemia is a risk factor for developing coronary artery disease. Lifestyle modification including an intake of healthy food as well as medication have approved effect in lowering serum cholesterol.

Objective: The primary objective of the present study was to determine the impact of a gamma-oryzanol-enriched rice bran oil, a product of Thailand, on serum cholesterol level.

Materials and Methods: A total of in 54 hypercholesterolemic patients were divided into two groups; RBOh (20,000 ppm of gamma-oryzanol, n = 27), and RBOn (5,000 ppm gamma-oryzanol, n = 27). The assigned RBO (15 ml) was intake each day for 8 weeks. Fasting serum lipids were measured at baseline and at the 4th and 8th weeks of the intervention. All patients were advised about lifestyle modifications.

Results: When compared to the baseline, subjects received RBOh showed a significant difference in 2 parameters including a reduction of cholesterol level at 8th weeks (*p*-value = 0.0101), and decrease in LDL-C level at the end of 8th weeks (*p*-value = 0.0013). In the group treated with RBOn, a significant increase in HDL-C level at the end of 8th weeks (*p*-value = 0.0303) without any effect on total cholesterol or LDL was observed. No sign of toxic effect on liver or renal functions was seen in both treatment groups.

Conclusion: RBO with gamma-oryzanol-enriched could decrease cholesterol and LDL-C level in hypercholesterolemic patients. Therefore, gamma-oryzanol-enriched RBO is a functional food that may reduce cardiovascular disease risk factor.

Keywords: Hypercholesterolemia, Rice bran oil, Gamma-oryzanol, *Oryza sativa*

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Cardiovascular disease (CVD) is the leading cause of morbidity and mortality globally[1]. It is largely caused by diet and lifestyle related factor. The common type of CVD is coronary heart disease (CAD). It is related with atherosclerosis; building up to plaque in the intima layer of artery vessel[2]. Dyslipidemia is a group of metabolic disease in which there are high lipid level over a prolong period and increase the risk of atherosclerosis, and leading to many complications in long term period such as myocardium infarction, ischemic stroke, and peripheral organ ischemia[1,2].

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Rice bran oil (RBO) is made from the pericarp and germ of the *Oryza sativa* seed. It is enriched with phytosterols, especially gamma-oryzanol and many other biologically active phytosterols. Gamma-oryzanol (γ-OR) is a mixture of 10 distinct ferulic acid (4-hydroxy-3-methoxycinnamic acid) esters of triterpene alcohols and sterols found in rice bran and its oil. 24-methylenecyclo-artanyl ferulate, cycloartenyl ferulate, campesteryl ferulate, and sitosteryl ferulate are the most abundant components generally accounting for approximately 80% of γ-OR in RBO[3]. However, the actual concentration of γ-OR in RBO may vary as it is affected by type of rice cultivar and extraction and processing conditions[4]. γ-OR has been associated with many beneficial effects including lipid lowering effect, antioxidant activity, enhanced learning and memory in mice, reduced blood pressure in rats, inhibited motility and invasion activity of liver and bile duct cancer cells and decreased fasting blood glucose and serum triglyceride in rats[5,6]. Hypocholesterolemic activities of RBO and γ-OR have been shown in various animal and human studies[7-21]. High serum γ-OR concentration was positively correlated with the reduction of serum lipid[16]. Rice bran oil derived from Thai brown rice by innovation technique, or virgin enriched rice bran oil, is the special rice bran oil which enriched with phytosterols and vitamin E, especially gamma-oryzanol and many other biologically active

phytosterols. The high concentration of γ-OR in Thai-RBO may lowering of cholesterol in the patients with hypercholesterolemia.

Objective

The aim of this study was to assess the cholesterol lowering effects of gamma-oryzanol-enriched rice bran oil comparison with cooking oil in the Thai patients with hypercholesterolemia.

Materials and Methods

Study design

This study was an 8 weeks, double-blind randomized clinical trial conducted from June, 2019 to April, 2020, at the Out-patient Department, Srinagarind hospital, Faculty of Medicine, Khon Kaen University, Thailand. The study protocol was approved by the Ethic Committee for Human Research, Panel 1, Khon Kaen University) (HE621060). All patients gave their written informed consent before enrollment in the present study. The present study was a phase 3 clinical trial. The primary outcome was the reduction of cholesterol level in the plasma, compared between 2 formulation of RBO, gamma-oryzanol-enriched; 20,000 ppm (RBOh) and 5,000 ppm (RBOn).

Study subjects

All patients were eligible if they met the following criteria; 1) male or female ages 20 to 60 years, and 2) serum cholesterol >200 to 250 mg/dL. Patients were excluded if; 1) high serum creatinine (>1.017 mg/dL in male, and >0.95 mg/dL in female), 2) alanine aminotransferase (ALT: 0 to 33 U/L), aspartate aminotransferase (AST: 0 to 44 U/L) more than 2.5 times above the upper normal level, 3) history of oil allergy, 4) poor control of systemic disease, such as stage III hypertension, fasting plasma glucose >250 mg/dL, HbA1C >8 mg%, current cardiovascular disease, and pancreatitis, 5) lactation or pregnancy, 6) current used of hypolipidemic drugs.

Study procedures

All subjected received a physical examination and blood sampling. The eligible patients were randomly assigned, using block of two, to RBOh (20,000 ppm of γ-OR), or RBOn (5,000 ppm of γ-OR) 15 ml daily for 8 weeks. There were informed regarding life-style modification and appointment visit at 4th and 8th weeks. All visit the patients come to the clinic for physical examination. Before starting the intervention (as baseline values) and at 4th and 8th weeks of the intervention, fasting plasma glucose (FPG), hemoglobin A1C, lipid profiles (triglycerides, total cholesterol, LDL-C and HDL-C), blood pressure (SBP, DBP and pulse rate), hepatic enzymes (aspartate transaminase and alanine transaminase) and creatinine levels were determined. In addition, at the end of the intervention, quality of life (QoL) was assessed by SF-36 questionnaires (Thai version).

Intervention

Gamma-oryzanol-enriched rice bran oil (RBOh) and cooking oil (RBOn) used in this study were manufactured by Medifoods company (manufacturing standard GMP NOP HACCP GREEN INDUSTRY, Bangkok, Thailand), each product prepared from the same lot number.

Treatment outcomes

The primary outcome of this study was decrease of serum cholesterol level from baseline to the end point. The secondary outcome variables were responds to the QoL and laboratory investigation.

Safety monitoring

Adverse reaction was assessing by physical examination and blood sample for determine liver and renal function test at the beginning and the 4th and 8th weeks of the study period. Each subject also completed the intervention with another sign and symptom that developed or become worse from baseline to the end of study.

Statistical analysis

All statistical analysis was performed using stata statistical software version 10 under the license of KKU. Sample size calculation, to obtain a 80% statistical power with an alpha error of 0.05 (one-side test), to demonstrated an effect a 15% decrease of cholesterol in patients post intake RBO, 23 patients were needed, assuming an overall loss follow-up of 15%, the subject number for each group were 27, and total subject number in the study were 54. The result variables were expressed as mean $\pm$ SE, mean difference and 95% confidence interval (95% CI). Data within group and between groups were compared using the student's pair t-test and ANOVA, respectively. Intent-to-treat analysis was performed on all effectiveness of patients who received at least on dose of RBO. Almost analysis used the level of statistical significant *p*-value <0.05.

Results

Demographic and other baseline characteristic of study patients

Characteristics of the study subjects were summarized in Table 1. A total of 54 eligible patients were randomly assigned to receive RBOh or RBOn formulas. 27 patients in RBOh group were 5 male, and 22 female, average ages of the subject was 53.29 years, and average BMI was 25.25. RBOn group were 5 male, and 22 female, average ages of the subject was 54.11 year, and average BMI was 24.47. After the start of the clinical study, 5 patients were withdrawn because of adverse reaction (3 patients suffered from nausea and vomiting; one patient in RBOh group, 2 patients from RBOn group, and two patients of unknown cause of loss to follow-up in both groups), 49 patients enrolled completed the clinical study, 25 in RBOh, and 24 in RBOn group.

Efficacy outcome

Effects of RBO on plasma lipid level in

Table 1. Baseline characteristic and demographic data of the patients

Variables	RBOh (n = 27)	RBOn (n = 27)
Age (years) (mean ± SE)	53.29±1.52	54.11±1.41
Gender male/female (n, %)	5 (18.5)/22 (81.3)	5 (18.5)/22 (81.3)
Body mass index ($kg.m^{-2}$) (mean ± SE)	25.25±0.51	24.47±0.69
Systolic blood pressure (mmHg ± SE)	134.78±3.74	133.33±3.29
Diastolic blood pressure (mmHg ± SE)	81.52±2.59	79.44±2.57
Heart rate (beat/min ± SE)	75.81±2.04	71.41±1.48
FPG (mg/dL ±SE)/HbA1c (mg% ± SE)	103.48±5.37/6.19±0.23	92.44±2.93/5.97±0.2
Creatinine (mg/dL) ±SE	0.86±0.03	0.86±0.03
ALT (U/L) ±SE	20.18±2.43	18.92±1.25
AST (U/L) ±SE	21.37±1.32	21.63±0.81
Smoking Habit (n, %)		
Non-smoking	26 (96.3)	27 (100)
Ex-smoker	0	0
Current smoker	1 (3.7)	0
Alcohol drinking (n, %)		
Non-drinking	22 (81.5)	17 (63)
Ex-drinking	5 (18.5)	10 (37)
Current drinking	0	0
Exercise (n, %)		
Non-exercise	6 (22.2)	5 (18.5)
Occasional	16 (59.3)	15 (55.5)
Habits	5 (18.5)	7 (26)
Underlying disease	9 (33.3)	10 (37)
DM	3	0
HT	2	2
Allergic rhinitis	3	2
Other; gout, PU, hypothyroidism, osteoporosis	1	6
Co-medication		
Sulfonylurea	1	0
Amlodipine	0	2
Enalapril	0	2
Colchicine	0	1

ALT = alanine transferase, AST = aspartate transferase, DM = diabetes mellitus, DLD = dyslipidemia, HT = hypertension, FPG = fasting plasma glucose, HbA1C = hemoglobin A1C

hypercholesterolemia patients were shown in Table 2 and Figure 1. When compared to the baseline, subjected received RBOh showed a significant differences regarding to 2 parameters including a reduction of cholesterol level at the end of 8^{th} (p-value = 0.0101) weeks, and a reduction of LDL-C at 8^{th} week (p-value = 0.0013). No significant effect on hemoglobin A1C, blood sugar, HDL-C and other observed parameters could be seen. In the group treated with RBOn showed a significant increase in HDL-C levels at the end of 8^{th} (p-value = 0.0303) weeks. No significant change could be observed in other observed parameters. During the period of intervention, no distinct side effect was reported, except some patients were withdrawn from the experiment since they had nausea and vomiting when eat oil.

Concerning the effect of the intervention on quality of life (QoL), all of them have high score of QoL, and no significant difference between groups. In addition, some positive subjective feelings such as gastrointestinal function, good quality of sleep, knee joint function, bright skin, and thicker of hair were recorded from either treatment groups.

Table 2. Effects of RBO on plasma lipid level in hypercholesterolemia patients

Variables	RBOh	RBOn
TC (mg/dL); baseline (week 0)	244±6.54	233.81±5.74
Post 4 weeks	236.11±6.43	229.37±6.73
Mean difference (w0 to 4)/ 95% CI	7.89±5.26/-2.93, 18.71	4.44±4.78/-5.37, 14.26
p-value	0.1461	0.3607
Post 8 weeks	231.7±5.06	232.07±6.69
Mean difference (w0 to 8), 95% CI	12.3±4.43/3.18,21.41	1.74±4.88/-8.3, 11.78
p-value	0.0101*	0.7245
TG (mg/dL); baseline (week 0)	122.89±10.58	129.59±11.55
Post 4 weeks	118.04±8.8	155.41±23.1
Mean difference (w0 to 4), 95% CI	4.85±7.71/-10.99, 20.7	-25.81±16.39/-59.51, 7.88
p-value	0.5346	0.1274
Post 8 weeks	140.89±16.08	136.44±13.61
Mean difference (w0 to 8), 95% CI	-18±10.59/-39.77,3.77	-6.85±8.96/-25.27, 11.56
p-value	0.1011	0.4513
HDL-C (mg/dL); baseline (week 0)	58.44±2.61	59.18±2.54
Post 4 weeks	59.33±3.02	60.33±2.9
Mean difference (w0 to 4), 95% CI	-0.89±1.12/-3.18, 1.41	-1.15±1.14/-3.49, 1.19
p-value	0.4336	0.3224
Post 8 weeks	57.56±2.94	62.22±2.86
Mean difference (w0 to 8), 95% CI	0.89±1.37/-1.93, 3.7	-3.04±1.32/-5.76, -0.31
p-value	0.5232	0.0303*
LDL-C (mg/dL); baseline (week 0)	189.56±7.39	171.41±6.91
Post 4 weeks	182.81±7.29	164.3±7.1
Mean difference (w0 to 4), 95% CI	6.74±5.47/-4.5, 17.98	7.11±4.17/-1.47, 15.69
p-value	0.2289	0.1004
Post 8 weeks	172.48±6.24	165.59±7.41
Mean difference (w0 to 8), 95% CI	17.07±4.73/7.35, 26.79	5.81±3.98/-2.38, 14.4
p-value	0.0013*	0.1566

TG = triglyceride; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol, TC = total cholesterol

Safety and tolerability

Adverse events (AEs) reported in 3 patients in RBO intervention were nausea and vomiting; 1 patient in RBOh group, and another 2 in RBOn group. At the end of the 8th weeks, two patients from both groups were lost follow-up for unknown reasons. There were no differences of ALT, AST, and creatinine level in any of treatment group. All reported AEs was mild in severity and self-limited solving. Overall, all patients were well tolerated and they were no clinical changes in blood chemistry or vital signs.

Discussion

This randomized clinical trial, gamma-oryzanol-enriched rice bran oil showed a significant reduction in both serum cholesterol and LDL-C level following 8-weeks intake. Our findings confirm that consumption of rice bran oil with high concentration of gamma-oryzanol can help lower both total cholesterol and LDL-C[8-11]. The previous reports showed a significant reduction of LDL-C (12.2%) after intake of 11,000 ppm gamma-oryzanol rice bran oil[21], and reduction of cholesterol (20 to 30%) after intake of gamma-oryzanol 50 mg/kg[22]. In the present study, cholesterol and LDL-C concentration were lowered by a relative change of 5.04% and 9.01%, respectively. Our findings confirm that consumption of gamma-oryzanol-enriched-rice bran oil can help lower both cholesterol and LDL-C. The hypocholesterolemic activity of γ-OR has been reported in the previous study suggests that γ-OR has a significant effect on lowering plasma cholesterol, by various mechanisms, including increase biliary secretion and fecal excretion of cholesterol[5,8,11], impaired apical uptake of cholesterol into enterocytes leading to decrease absorption in the

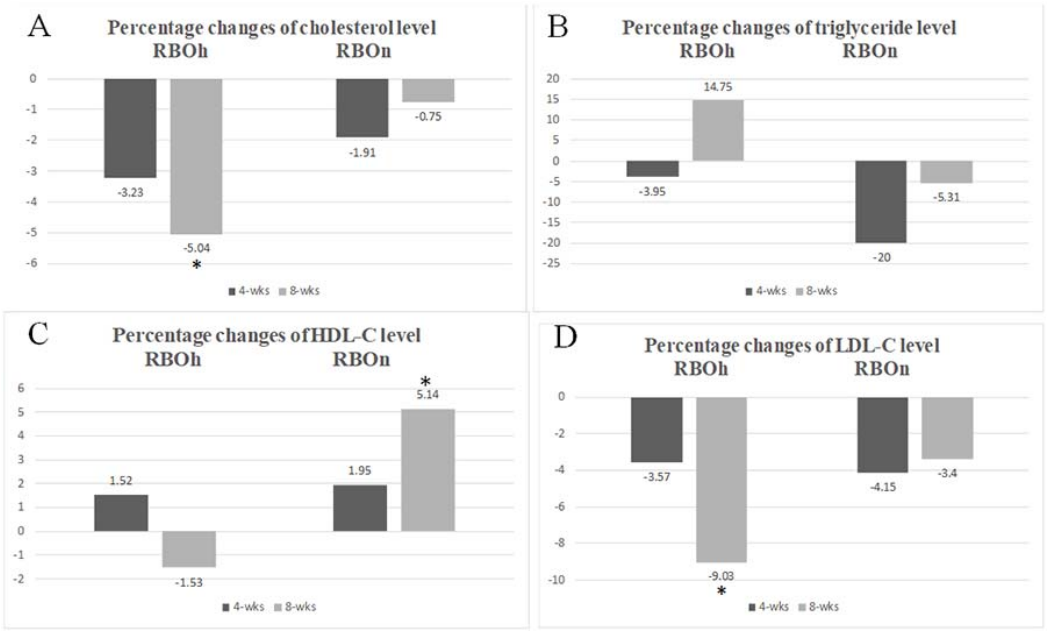

Figure 1. Percentage changes of the lipid parameters, include cholesterol (A), triglyceride (B), high density lipoprotein cholesterol (HDL-C) (C), and low density lipoprotein cholesterol (LDL-C) (D) of RBOh, and RBOn at the 4th and 8th weeks post intervention.

intestine[14,15], and inhibiting hepatic cholesterol synthesis via inhibited 3-hydroxy-3-methylglutary-coenzyme A (HMG-CoA) reductase activity[14]. There have been several study focused on the role of gamma-oryzanol to reduce lipid in the serum, because it was found lipid lowering effect after feeding gamma-oryzanol in rats. The researcher detected an accumulation of gamma-oryzanol; main active compound of rice bran oil, and it metabolite (ferulic acid), related with the reduction of serum lipid[6,15,16]. Moreover, many study concluded that rice bran oil might play a role to reduction in lipid level. After intake RBO, they founded a significant reduction in cholesterol absorption, alteration of lipid metabolism modulation, and decreasing of lipid synthesis[7,14]. These results were leading to reduction of total cholesterol, LDL-C, apolipoprotein, triglyceride, and induction of HDL-C[7,9]. In present study, low concentration gamma-oryzanol-rice bran oil has more increases HDL-C effect than rice bran oil containing high concentration of gamma-oryzanol. In addition, the serum triglyceride concentration of patients enrolled in this trial were in normal range that might not have been affected by the intervention.

Conclusion

The results of this study indicated that rice bran oil derived from Thai brown rice by innovation technique with high concentration of gamma-oryzanol could be useful as a product to improve health in general population, especially hyperlipidemia group who having high blood cholesterol and LDL-C level, while low concentration of gamma-oryzanol could increase HDL-C. In addition, these rice bran oil derived from Thai brown rice might help control and reduce the risks of many diseases linked with metabolic syndromes.

What is already known on this topic?

RBO has been lowering of total cholesterol, triglyceride, LDL-C, and increase HDL-C in animal and human. This study is the first study investigating the cholesterol-lowering of a rice bran oil derived from Thai brown rice by innovation technique, comparison of cooking oil (gamma-oryzanol 5,000 ppm) and gamma-oryzanol-enriched rice bran oil (gamma-ryzanol 20,000 ppm) in the patients with hypercholesterolemia who were undergoing life-style modification.

What this study adds?

The result showed that RBO derived from Thai rice with high concentration of gamma-oryzanol (20,000 ppm) could reduction of cholesterol and LDL-C in hypercholesterolemic patients. However, 5,000 ppm gamma-oryzanol- RBO might increase serum HDL-C. A rice bran oil derived from Thai brown rice by innovation technique might be used as an alternative medicine or functional food in the hypercholesterolemic patients who were undergoing with

life-style modification and diet control.

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Conflicts of interest

The authors declare no conflict of interest.

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