Asian Journal of Health Research
Journal Homepage: https://a-jhr.com
Published by Ikatan Dokter Indonesia Wilayah Jawa Timur

Original Research

Black-Eyed Pea Diet Alleviates High-Density Lipoprotein Cholesterol in Menopausal Women; Randomized Clinical Trial Study

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ARTICLE HISTORY
Received: 21 January 2022
Revised: 26 January 2022
Accepted: 22 February 2022

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KEYWORD
Black-Eyed Pea Diet; Isoflavone; Lipid Profiles; The Menopausal Woman

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ABSTRACT

Introduction: Menopause is defined as the last menstruation cycle in women. Estrogen concentration in menopausal women was decreased and altered the lipid metabolism profiles. Menopausal women reportedly have a high risk of cardiovascular disease, arthritis, diabetes mellitus, and stroke. This study focused on the effect of the black-eyed pea diet on the lipid profiles in menopausal women.

Methods: A randomized clinical study was performed to analyzedd the effect of the black-eyed pea diet on lipid profiles. Fifty-four menopausal women were selected as respondents and classified into two groups, black-eyed pea diet and placebo. Black-eyed pea diet treatment was performed by consuming black-eyed powder containing 67.5 mg of isoflavones and were monitored in week – 0 and week – 12 after treatment. Lipid profile of black-eyed pea diet and placebo in 12 weeks monitoring showed no significant value. After 12 weeks of treatment, total cholesterol and triglyceride were higher than before treatment.

Results: Interestingly, the black-eyed pea diet increased the HDL and lowered the LDL after 12 weeks of treatment.

Conclusion: This study concluded that black-eyed pea improved lipid profile, especially in elevating HDL level and decreasing LDL level in 12 weeks consumption.

Cite this as: Wiradharma H, Dwijasa PM, Maharani M. (2022) Black-Eyed Pea Diet Alleviates High-Density Lipoprotein Cholesterol in Menopausal Women: Randomized Clinical Trial. *Asian J Heal Res*. 1(1):30-35. doi: https://doi.org/10.55561/ajhr.v1i1.12

INTRODUCTION

Cardiovascular disease (CVD) is a group of diseases, including coronary heart disease (CHD), coronary artery disease (CAD), and acute coronary syndrome (ACS) [1]. In the U.S, coronary heart disease is the leading cause of death. The American Heart Association (AHA) data reported that in 2016, the overall death rate from CHD was 102.6 per 100,000 [2]. It also led to stroke to 2 million females, with 200.000 of them dying because of the disease. Menopause women dominate the death case of coronary heart disease in women. Every year, there are 1,5 million cases in females, with 500.000 of them at risk of death.

Premenopausal women have a low risk of cardiovascular disease [3]. At the same age, the cases of cardiovascular disease in men were eight times higher than in premenopausal women [3]. However, the risk of cardiovascular disease in women would be increased in the age of menopause. It is caused by no estrogen hormone produced after menopause [1,4,5]. After menopause, the exhaustion of ovarian follicles would lead to estrogen reduction. Premenopausal women have higher Nitric Oxyde levels that could protect the heart and inhibit smooth muscle proliferation in heart disease [6]. In post-menopausal women, the free fatty acid is highly increased due to a significant reduction in estrogen. It makes post-menopausal women have a high chance of developing metabolic syndrome and insulin

resistance, which are considered the risk factor of CHD [1,7–9].

Regardless of the importance of estrogen, the condition in postmenopausal women is different. The estrogen in menopausal women was decreased, and it also changed the lipid profile [5,10,11]. The high level of Low-Density Lipoproteins (LDL) and the low level of High-Density Lipoprotein (HDL) will increase the risk of coronary heart disease in women [9,10]. The HDL and LDL levels possibly influence the risk of cardiovascular disease. Hormone replacement therapy (HRT) could be applied To reduce the symptoms associated with menopause [12]. The treatment includes an estrogen and progesterone component to mimic the natural hormones created by the ovary [1,12,13]. Experts accept HRT as the primary preference that could decrease the case of cardiovascular heart disease. It is advantageous in reducing the case rate of coronary heart disease. Contrary to the clinical study by Heart and Estrogen-progestin Replacement Study (HERS), it mentioned that the random clinical study to 2763 menopause women with conjugated equine estrogen (CEE) and medroxyprogesterone acetate (MPA) had no effect in reducing the case rate of coronary heart disease as a secondary preference [5].

Despite the advantage, HRT developed certain risks and adverse effects. Mastodynia, fluid retention, nausea may occur during the usage of estrogen [12]. The double-blind, randomized clinical study from Women's Health Initiative (WHI) showed that in 5 years (1997-2002), 16,608 women with HRT therapy had a higher risk of getting sick than placebo treatment. This research used conjugated equine estrogen (CEE) 0.625 mg/day and medroxyprogesterone acetate (MPA) 2.5 mg/day compared to placebo treatment of 50-79 years older women. Women who experienced HRT had a 2.13 times higher risk of lung embolic and 1.42 times higher risk of stroke than placebo [1]. Long-term use of HRT increases the risk of breast cancer by 10-30% [12].

Considering the high side effects, it leads to the discovery of estrogen-like compounds with low side effects, such as phytoestrogen. A study mentioned that phytoestrogen is a natural agent for HRT and is high safety for consumption [13]. Phytoestrogen is found in beans, showed similar structure with 17-β-estradiol (E2 [8,14]. PERMI Malang found isoflavone in local plants in Malang, such as jicama, cowpea, and aloe vera. Black-eyed pea also reported high isoflavone with a similar structure to natural estrogen. Plant phytoestrogen reported reduced menopausal symptoms, decreased cardiovascular disease, metabolic syndrome, and breast cancer risk factors [8,14]. The effect of the black-eyed pea diet on dyslipidemia in post-menopausal women has remained unclear. Therefore, the current study evaluated the impact of black-eyed pea supplementation on dyslipidemia of post-menopausal women.

MATERIAL AND METHODS

Clinical Experiment

Sixty respondents from Kauman – Klojen, Gadingkasri – Klojen, Gadang – Sukun, Karang Besuki – Sukun, Bunul Rejo – Blimbing, Polehan – Blimbing, Sawojajar – Kedung Kandang, Buring – Kedung Kandang, Tlogomas – Lowokwaru, and Tulusrejo – Lowokwaru were selected in this study. Respondents who approved research informed consent were interviewed and divided into black-eyed pea diet and placebo. All respondents were menopausal women with dyslipidemia and not fulfilled the exclusion criteria. The exclusion criteria included using hormone therapy, consuming anti-lipid drugs, performing diabetes mellitus, breast cancer, endometriosis, and sensitivity with estrogen.

Lipid Profile and Blood Glucose Assessment

Total cholesterol, triglyceride, HDL, LDL, and blood glucose were monitored in weeks 0 (before treatment) and 12 (after treatment). Total cholesterol of human blood sera was analyzed by colorimetric test. The reaction mix consisted of 10 µL sample in 1000 µL total cholesterol reagent (0.25 mmol/L 4-aminophenazone, phenol, 5.0 U/ml peroxidase, 0.15

Table 1. Clinical Profile of Black-Eyed Pea Diet and Placebo Group

Variables	Black-Eyed Pea Diet	Placebo
Age (average)	60 years and 25 days	60 years and 13 days
Menopause periods (average)	11 years	10 years, 8 months, 12 days
Obesity		
Yes	3	3
No	24	24
Physical activity		
Yes	4	5
No	23	22
Fat Diet		
High	8	9
Low	19	18

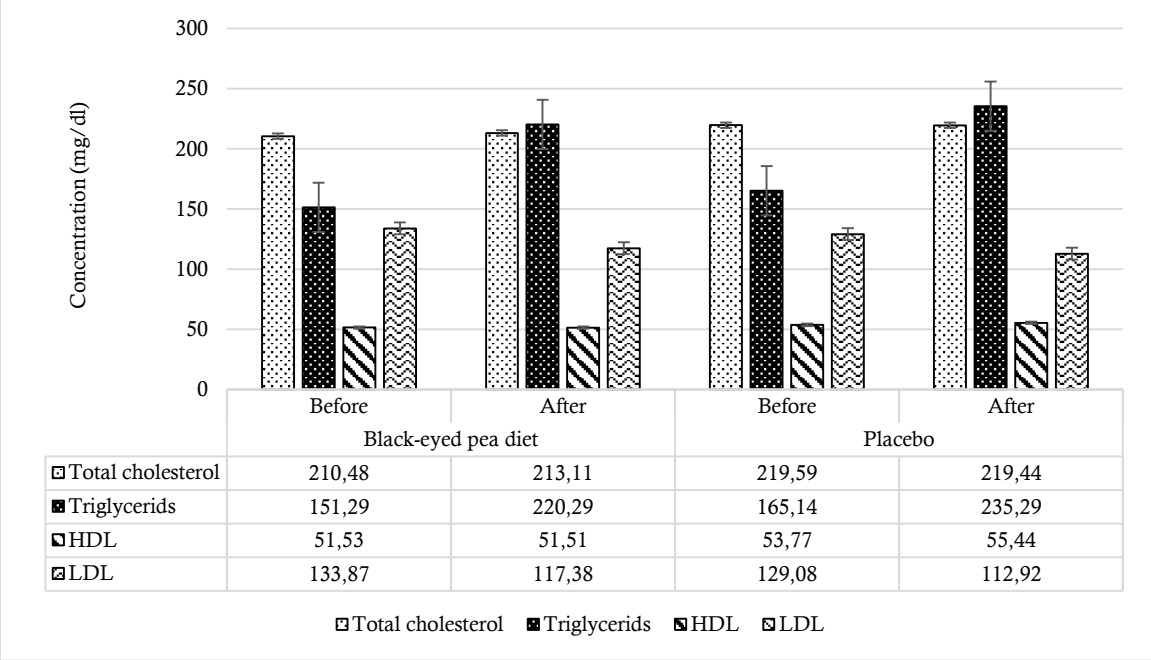

Fig. 1. Lipid Profiles of Black-Eyed Pea Diet and Placebo

U/ml cholesterol esterase, 0.1 U/ml cholesterol oxidase, buffer dan stabilizer) was incubated for 10 mins in 37°C and measured by Ceretium NB-201 Semi-Auto Chemistry Analyzer. Triglyceride (TGA) was measured by mixing 10 µL blood sera with triglyceride reagent (5.0 mM magnesium sulfate, 0.7 mM 4-Aminoantipyrine, 5.0 mM m-hydroxybenzoic acid, 7000 U/l glycerylphosphate oxidase, 0.01 % sodium azide, 200.000 U/l lipases, 1000 U/l glycerol kinase, 2000 U/l peroxidase, and 50 mM buffer) up to 1mL reaction mix. The reaction mix was incubated at 37°C for 10 minutes and analyzed by Ceretium NB-201 Semi-Auto Chemistry Analyzer. High-density lipoprotein (HDL) was conducted by HDL precipitation. Blood sera 500 µL in Stanbio HDL Liquicolor (1185 mmol/l magnesium sulfate, 1.1 % dextran sulfate, and stabilizer) was centrifuged at 4000 rpm for 10 mins to collect the supernatant. Ceretium NB-201 Semi-Auto Chemistry Analyzer analyzed the supernatant. The following formula calculated Low-density lipoprotein of blood sera:

$$LDL = Total\ Cholesterol - (HDL + \frac{TGA}{5})$$

Data Analysis

A randomized clinical study was used in this study. Data were analyzed by analysis of covariance (ANCOVA) with a significant value $p<0.05$.

Ethics

This study was approved by local Institutional Review Board, and all participants have provided written informed consent prior to involvement in this study.

RESULT

Characteristics of Subject

Fifty-four out of 60 women were selected as respondents, consisting of 27 respondents with the black-eyed pea diet and placebo. The characteristic of respondents were described in Table 1. The average of respondents ages both of black-eyed pea diet and placebo approximately 60 years old, and the respondents had more than ten years menopause periods. Respondents with or without black-eyed pea diet showed three were obese, had low physical activity, and 8 – 9 of them consumed a high-fat diet.

The Effect of Black-Eyed Pea Diet on Dyslipidemia

The profiles of lipid total in the black-eyed pea diet and placebo were variable (Fig. 1). Black-eyed pea increased the total cholesterol and triglycerides and performed lower total cholesterol and triglyceride levels

Table 2. Comparative Analysis of The Research Variable to Total Cholesterol, Triglyceride, HDL, and LDL Change

Variables	Cholesterol total		Triglyceride		HDL		LDL		Dyslipidemia change	
	Mean	*p* value*	Mean	*p* value*	Mean	*p* value*	Mean	*p* value*	Mean	*p* value*
Age	420.18	0.48	2255.76	0.63	110.93	0.16	6.29	0.91	0.45	0.09
Menopause	237.80	0.60	16066.44	0.21	69.69	0.27	15.35	0.86	0.31	0.15
Obesity	2065.13	0.13	8958.15	0.34	6.69	0.73	409.82	0.38	0.35	0.13
High-fat diet	1291.88	0.23	6751.84	0.41	2.37	0.84	159.70	0.59	0.13	0.76
Activity	1972.70	0.14	470.86	0.83	127.96	0.13	1485.92	0.10	0.209	0.24
Group (Black-eyed pea diet and placebo)	19.10	0.88	9383.90	0.33	51.78	0.34	6.32	0.91	0.24	0.21

* *p value<0.05*

than placebo. In HDL, the black-eyed pea diet stabilized the HDL concentrations and decreased the LDL levels. Placebo treatment showed higher triglyceride and HDL levels after 12 weeks of observations. Not significantly effects of black-eyed pea diet might be influenced by obesity condition, low physical activity, and consuming high-fat diet among respondents.

Comparative Analysis of Total Cholesterol, Triglyceride, HDL, and LDL Change in Subject

The comparative analysis in total cholesterol, triglyceride, HDL, and LDL was represented in Table 2. Age, menopause, obesity, high-fat diet, activity, and black-eyed pea diet were not significantly correlated with lipid profiles and dyslipidemia (Table 2). In the total cholesterol, all confounding variable had $p > 0.05$ (age = 0.489, menopause = 0.602, obesity = 0.129, high fat diet = 0.227, and activity = 0.137). In addition, the cholesterol total in the treatment group also showed no significant effect (p = 0.882). The p score of all confounding variables were $p > 0.05$ (age = 0.633, menopause = 0.206, obesity = 0.344, high fat diet = 0.410, and activity = 0.827). The black-eyed pea powder treatment also did not significantly affect the triglycerides level, with p= 0.332. The effect of confounding variables on LDL level was similar to HDL and triglycerides level, which did not show a significant effect ($p > 0.05$). Variable age had p= 0.914, menopause had p= 0.867, obesity had p =0.387, a high-fat diet had p 0.588, and activity had p= 0.103. However, the p score of the treatment group had a score of 0.914.

DISCUSSIONS

Black-eyed pea powder containing 67.5 mg isoflavone did not significantly improve lipid profiles in 27 menopausal women for 12 weeks diet. However, HDL of black-eyed pea respondents was stabilized, and LDL showed lower concentration than before the black-eyed pea diet. Menopause is the last period of menstruation, and it occurs at the age of 51 years old [5,8,10]. Several metabolisms were changed in menopausal women, including a lower concentration of estrogen, low HDL level, and high total cholesterol, triglycerides, LDL, and VLDL concentration in blood plasma [15–18]. Lipid profiles change in menopausal women was affected by decreasing estrogen levels. Low estrogen elevated nitrous oxide and reduced vascular permeability. Estrogen is essential for endothelial cell stabilizers, antioxidant stimulating activity, and fibrinolysis protein alteration. Low estrogen altered lipid profiles and stimulated a high risk of cardiovascular diseases [7,11,19]. Besides that, menopausal periods also reported reducing physical activity in Nepal women and increased cardiovascular risk [4]. Several treatments in nutrition, synthetic supplements, and natural compounds have been identified for antiobesity repurposing treatments.

Feldman *et al.* (2021) reviewed that plant polyphenols attenuated lipid disorders, ameliorated lipid metabolism, and alleviated hyperlipidemia *in vitro* and *in vivo study.* several nutrients also reported improving lipid profiles. Omega-3 fatty acids were a PPARα agonist and inhibited lipid production. Dietary fibers also delayed cholesterol absorption, monacolin inhibited the activity of the HMG-CoA receptor.

Phytosterol was reported that inhibit cholesterol absorption [14,15,17–20]. Anthocyanins from black rice also ameliorated hyperlipidemia based on *in vivo* and *silico* studies [21–24]. Previous research reported that an isoflavone from lactic-acid fermented soy milk lowered lipid gene metabolism expression in rats fed a high cholesterol diet [17]. A clinical study also reported that soy isoflavones did not affect serum triglycerides, LDL, and total cholesterol in peritoneal dialysis patients [9,25]. However, in HDL concentration, soy isoflavones elevated 11.5% of HDL in peritoneal dialysis patients [25].

CONCLUSION

Menopausal women naturally have low physical activity and alter lipid metabolism profiles due to the low estrogen concentration. The black-eyed pea diet did not significantly ameliorate lipid profiles in menopausal women, but it stabilized the HDL concentration in blood plasma for 12 weeks. This research suggested that consuming high isoflavones in black-eyed pea powder might prevent menopause and improve lipid metabolism profiles in postmenopausal women.

ACKNOWLEDGMENTS

We thank anonymous referees for their valuable suggestions.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest regarding the publication of this article.

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