Indonesian Journal of Science & Technology

Journal homepage: http://ejournal.upi.edu/index.php/ijost/

Hypocholesterolemic Activity of Kedawung (*Parkia roxburghii*)

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ABSTRACT

The purpose of this study is to investigate the hypocholesterolemic activity of kedawung (*Parkia roxburghii*), an underutilized and lesss-known tree nut. Kedawung was prepared into kedawung powder (BK), kedawung extract (SK) and hydrolyzed kedawung (HK) and then analyzed for its proximate and total phenol content. Furthermore, SK and HK were administered to rats to evaluate their effects on the lipid profile. Oral administration of HK at 160 mg/kg body weight increased the HDL cholesterol level and lowered the LDL and total cholesterol levels in blood of the administered rats. Among groups of the administered rats that completed the 35-day treatment, total cholesterol and LDL cholesterol were significantly reduced (p<0.01). The present data showed that kedawung has a hypocholesterolemic activity because of its phenolic content that acts as antioxidant that can bind cholesterol in blood; composition and distribution of amino acid in HK that changed the cholesterol metabolism (reduced cholesterol concentration); protein content in HK that increased the production of lipoprotein that plays a role in suppression of plaque and atherosclerosis. Therefore, hydrolyzed kedawung can be used as an hypocholesterolemic food material.

ARTICLE INFO

Article History:
Submitted/Received 12 Nov 2020
First revised 23 Mar 2021
Accepted 20 May 2021
First available online 28 May 2021
Publication date 01 Sep 2021

Keywords:
Kedawung,
Hypocholesterolemic.

1. INTRODUCTION

Cardiovascular diseases are the major reason of death in the world, and taking an estimated 17.9 million each year, representing 31% of all global deaths. Of this portion, the number of people who suffer from cardiovascular disease or have a high cardiovascular risk such as hypertension, diabetes, and hypercholesterolemia reaches 64.3%. Most cardiovascular diseases can be prevented by addressing behavioural risk factors such as tobacco use, unhealthy diet and obesity, physical inactivity and excessive alcohol intake. The risk of cardiovascular disease can be reduced by controlling and lowering the cholesterol level in blood by consuming hypocholesterolemic foods in daily diet (Adams *et al*., 2004).

One of less known, underutilized, and nutritious leguminous plants which have a potential as a food material to produce hypocholesterolemic food is kedawung (*Parkia roxburghii*). Kedawung is an endemic plant from Meru Betiri National Park (Angami *et al*., 2017).

Fitriyana, (2017) has produced hypocholesterolemic foods from edamame (*Glycine max*) lowering total cholesterol of administered rats. Hypocholesterolemic food is needed as an effort to suppress and prevent the increase of cardiovascular diseases. Therefore, this study aims to investigate the hypocholesterolemic activity of kedawung.

2. MATERIALS AND METHODS

2.1 Sample preparation

Kedawung pods were collected from Meru Betiri National Park, Jember, Indonesia. All the pods were scrapped to separate them from outer peel and soaked for 24 h with 6 times water replacement. Then, kedawung pods were roasted until the seeds were separated from its peel. Kedawung powder (BK) was obtained after drying for 5 hours at 50°C then mashed and passed through a sieve with 40 mesh. Kedawung extract (SK) was obtained by suspending kedawung powder in distilled water with a ratio of 1 : 6 (w/v). Hydrolyzed kedawung (HK) was obtained by enzymatical hydrolyzation of SK using thermolysin, a hydrophobic protease. SK and HK were administered to rats at the doses of 160 mg/kg body weight (BW) and 320 mg/kg BW for 35 days.

2.2 Total phenol content

Total phenol content was analyzed by following the procedure suggested by (Blainski *et al*., 2013). Fifty µL of the Folin-Ciocalteu reagent (50%) was added to 1 mL of Na_2CO_3 (7.5%) and 450 µL of solution of tested sample. The mixture was kept for 30 min at room temperature. The absorbance was measured at a wavelength of 725 nm. The absorbance values obtained from each tested solution were put in the regression equation of standard solution of gallic acid made with concentration of 0 µg/mL, 50 µg/mL, 100 µg/mL, 150 µg/mL, 200 µg/mL, and 250 µg/mL.

2.3 Hypocholesterolemic effect

Wistar strain male white rats (*Rattus norvegicus*) were previously adapted for 7 days (aclimatization) at the Clinical and Pathological Laboratory, Faculty of Pharmacy, University of Jember, Indonesia. Standard feed was given at 20 gram/rat and drinking water was given in ad-libitum. SK and HK were orally given at the doses of 160 mg/kg BW and 320 mg/kg BW.

2.3.1 Treatment of experimental animals

After adaptation, rats were randomly divided into 6 groups of 6. Each group was treated once for 35 days.

On the 36th day, about 3.0 mL of blood from the tail was collected in Eppendorf tubes. The tube was kept for 3 h and centrifuged at 3,000 rpm for 15 min. The serum was taken and kept in the refrigerator.

2.3.2 Determination of total cholesterol

DOI: https://doi.org/10.17509/ijost.v6i2.34544
| p- ISSN 2528-1410 e- ISSN 2527-8045 |

One mL of Fluitest-CHOL cholesterol reagent solution (Analyticon, Lichtenfels, Germany) composed of 90 mM PIPES buffer pH 6-9, 26 mM Phenol, 200 U/L of cholesterol oxidase, 300 U/L of cholesterol esterase, 1250 U/L of peroxidase, 0.4 mM 4-aminoantipyrine, 5.17 mM cholesterol and 10 µL of sample was mixed using vortex and kept for 5 min at 37°C.

Absorbance was measured at a wavelength of 500 nm. Mixture of 1000 µL of cholesterol reagent and 10 µL of distilled water was used as a blank. Measurement of total cholesterol concentration is described below:

$= \frac{\Delta A\ sample}{\Delta A\ standard} x\ standard\ concentration$

Standard concentration: 5.16 mM (200 mg/dL)

2.3.3 Determination of HDL-cholesterol

Sample (200µL) was mixed with 500 µL of precipitator solution (0.55 mM phosphotungstic acid and 25 mM magnesium chloride), and then shook and kept for 10 min at 15-25°C. The mixture was then centrifuged at 4,000 rpm for 10 minutes. The supernatant (100 µL) was mixed with 1,000 µL of reagent cholesterol, homogenized with vortex and kept for 20 min at room temperature. Then the absorbance at 500 nm was measured. Measurement of HDL-cholesterol level is described: $\Delta Asample\ x\ 220$.

2.3.4 Determination of LDL-cholesterol

100 µL of sample was mixed with 1,000 µL reagent LDL (0.68 g/L of heparin; 64 mM sodium citrate; 2% stabilizers (hitergent: 5% ethanolamine pH 12.5) and then shook and kept 10 min at 15-25°C. The mixture was then centrifuged at 4,000 rpm for 15 minutes. The supernatant (100 µL) was mixed with 1,000 µL of reagent cholesterol, homogenized with vortex and kept for 5 min at 37°C. Then, the absorbance at 500 nm was measured.

Measurement of LDL-cholesterol concentration is described:
= concentration of total cholesterol-$(\Delta Asample\ x\ 690)$.

2.4 Statistical analysis

Data obtained were expressed as mean ± standard deviation (SD). Oneway ANOVA followed by Tukey-Kramer test was used to assess the statistical significance of the difference against control.

3. RESULTS AND DISCUSSION

3.1 Total phenol content

Total phenol contents in kedawung seed preparations are indicated in the following **Table 1.**

SK has the lowest content of total phenol, since it was prepared from BK roasted for 20 min and diluted with distilled water. Drying process could influence the existence of bioactive compound in food materials. Polyphenols will decrease in heating process. HK has higher content of total phenol than SK, but lower than BK. HK was obtained through enzymatic hydrolysis of BK. Protein hydrolysis of kedawung by enzyme produced peptide fragments, and it was expected that it contains anti-cholesterol bioactive peptides.

Table 1. Total phenol in kedawung seed preparations.

Products	Absorbance	Total Phenol (mgGAE/g sample)	Average (mgGAE/g sample)
BK	0.448	0.324	0.134 ± 0.055
SK	0.138	0.023	
HK	0.173	0.057	

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p- ISSN 2528-1410 e- ISSN 2527-8045 |

Kedawung is rich in protein content (29-32%; mostly consist of albumin and globulin) (Longvah *et al*., 1998), essential amino acids (isoleucine, leucine, phenylalanine, and tyrosine), and fatty acids (oleic and linoleic acids) (Angami, *et al*., 2017; Dajanta *et al*., 2011). Processing methods influence nutritional and anti-nutritional contents (Salam *et al*., 2014). Sathya and Siddhuraju, (2015) have reported that protein (15-36%) and lipid contents (11-69%) in kedawung seed are enhanced after some processing.

Fatimah *et al*., 2019 have reported that most of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, and terpenoid have been extensively used in the preparation of medicinal industry. High phenolic content provides physiological and nutritional benefits, and many studies have shown its strong inverse correlations with risk of cardiovascular diseases (Fatimah *et al*., 2018). Natural food fiber, phytosterol, and polyphenol in kedawung are able to bind cholesterol in blood. Polyphenol in kedawung acts as an antioxidant that is beneficial for the health of human body by decreasing heart disease attack, hypertension, osteoporosis and cholesterol (Ponnusha *et al*., 2011; Samruan *et al*., 2012). (Masuda *et al*., 2017) also have reported that polyphenols as antioxidants prevent various diseases associated with oxidative stress, such as cardiovascular diseases.

3.2 Hypocholesterolemic effect

Lipid profiles of serum from rats are indicated in **Table 2**. Level of total cholesterol of administered rats increases along with increase in sample concentrations of SK and HK. Meanwhile, LDL and HDL cholesterol levels increase along with increase in sample concentrations. HK influences the lipid profiles of rats.

Nutrients and bioactive compounds in kedawung influence the lipid profiles, especially the decrease in LDL cholesterol. These components are protein, fiber, vitamins, unsaturated fatty acids, and flavonoids. The main peptide components in kedawung protein are β-conglycinin, 7S globulin, glycinin and 11 globulin. The peptide could increase the activity of LDL receptors and degrade LDL in liver cells. This leads to the decrease in the LDL cholesterol level in serum (Adams et al., 2004).

According to **Figure 1** and **Figure 2**, SK significantly stimulates the HDL cholesterol level in serum at 320 mg/kg BW. However, 160 mg/kg BW of SK does not affect the HDL cholesterol levels. In addition, HK also statistically significant stimulates the HDL cholesterol levels at 160 and 320 mg/kg BW. However, either SK or HK does not affect the LDL cholesterol level in serum.

Table 2 Lipid profiles of serum from administered rat.

TREATMENT	TC (mg/dL)	LDL (mg/dL)	HDL (mg/dL)
Control	44.44	33.44	30.89
Negative Control	67.75	55.85	27.60
SK at 160 mg/kg BW	57.77	49.02	25.71
SK at 320 mg/kg BW	60.85	49.96	40.43
HK at 160 mg/kg BW	49.95	54.96	45.13
HK at 320 mg/kg BW	50.06	52.08	56.75

DOI: https://doi.org/10.17509/ijost.v6i2.34544 |
p- ISSN 2528-1410 e- ISSN 2527-8045 |

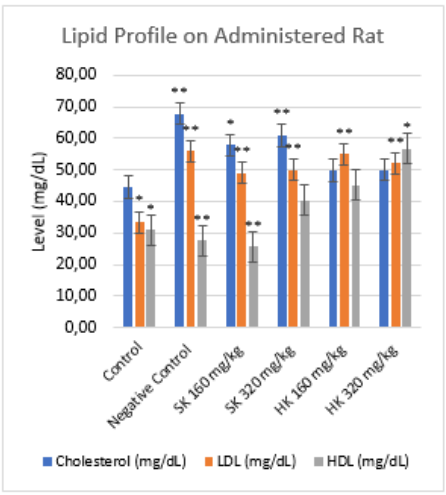
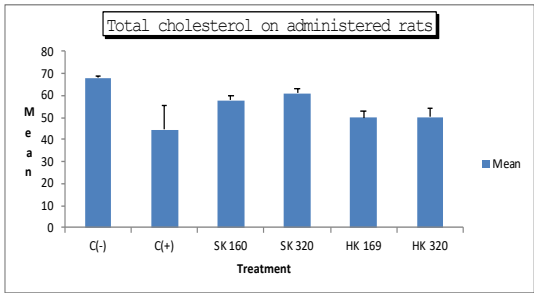
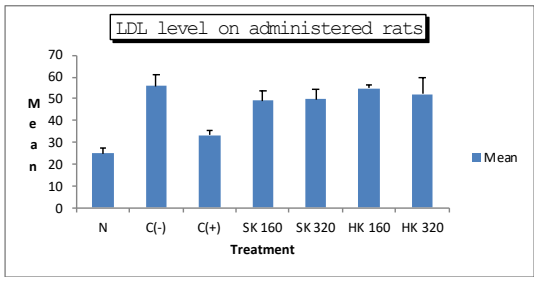


Figure 1. Lipid profile of administered rats.

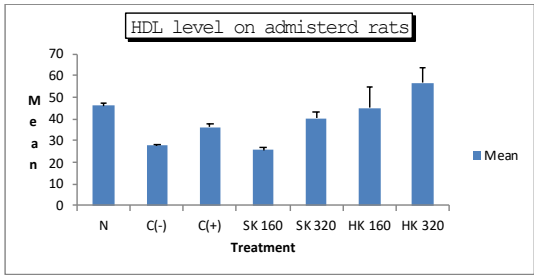
Data are represented as mean ± standard deviations (n = 6). *p<0.05 or **p< 0.01 against control by Tukey-Kramer test.



(a)



(b)



(c)

Figure 2. (a) Total cholesterol; (b) LDL level; (c) HDL level on administer rats.

DOI: https://doi.org/10.17509/ijost.v6i2.34544|
p- ISSN 2528-1410 e- ISSN 2527-8045 |

The decrease in total cholesterol in SK group may be due to some ingredients contained in kedawung. (Zhong *et al*., 2007) reports that poly-unsaturated fatty acid (PUFA) has the ability to lower the LDL cholesterol levels since it can increase the number of LDL receptor and decrease VLDL secretion from liver. Proteins contained in kedawung are able to decrease the absorption of cholesterol from intestine and reabsorption of bile acids that could cause an increase in the secretion of neutral sterol and bile acids in feces. Kedawung contains a mixture of phospholipid complex of 50-97% most of which is phosphatidylcholine (76%) and phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine. Choline as part of lecithin is known to be essential to prevent fat accumulation in liver since it plays an important role in fat metabolism, and lecithin could dissolve fat and secrete it out the body (Mourad *et al*., 2009). In addition, vitamin B_3, which is contained in kedawung, can lower the production of VLDL in liver. As an effect, the production of total cholesterol and LDL also decreases. Calcium could bind to bile acid in small intestine by which insoluble calcium complex of bile salts can be formed and secreted through feces. The excretion of calcium complex with bile acid causes a decrease in the re-absorption of cholesterol (Zhong *et al*., 2007).

Mateos-Aparicio *et. al*., (2008) also reveal that the content of natural food fiber in kedawung is able to bind to excess cholesterol of experimental animals. However, the mechanism of kedawung as an anti-cholesterol substance has not been uncovered yet. It is likely due to four things. First, composition and distribution of amino acids in soybean changed the cholesterol metabolism thus reduces the concentration of cholesterol in blood. Second, proteins in kedawung increase the production of lipoprotein A that plays a role in suppression of plaque and atherosclerosis. Third, food fiber in kedawung lowers cholesterol in blood because the fiber binds to cholesterol and along with the fermentation of food fiber in large intestine, produced propionic acid lowers the synthesis of cholesterol. Fourth, polyphenols prevent the production of oxidated LDL, and reduce the risk of atherosclerosis or plaque accumulation in blood vessel wall.

Thermolysin used in the functional food production is an endopeptidase that breaks the peptide bonds at the inner part of peptide chains. The enzyme may hydrolyze proteins in kedawung extract into bioactive peptides. The enzyme is hydrophobic, and the hypocholesterolemic bioactive peptides generally have hydrophobic amino acids in its N terminal such as leucine in sequence of Leu-Pro-Tyr-Pro-Arg (Yoshikawa *et al*., 2005); Leu-Pro-Tyr-Pro dan Leu-Pro-Tyr-Pro-Arg (Yoshie-Stark *et al*., 2004) and tryptophan in sequence of Trp-Gly-Ala-Pro-Val-Thr, Trp-Gly-Ala-Pro-Ser-Leu (Gibbs *et al*., 2004), and Trp-Gly-Ala-Pro-Ser-Ile (Zhong *et al*.,2007).

4. CONCLUSION

In conclusion, it is indicated that HK (hydrolyzed kedawung) increases serum HDL cholesterol levels at 160 mg/kg body weight and lowers the LDL and total cholesterol levels. Administration of kedawung for 35 days significantly reduces total cholesterol and LDL cholesterol. The data suggest that kedawung has hypocholesterolemic activity. Therefore, kedawung is a hypocholesterolemic food material which can reduce the risk of cardiovascular disease.

5. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. Authors confirmed that the data and the paper are free from plagiarism.

DOI: https://doi.org/10.17509/ijost.v6i2.34544 |
p- ISSN 2528-1410 e- ISSN 2527-8045 |

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DOI: https://doi.org/10.17509/ijost.v6i2.34544|
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DOI: https://doi.org/10.17509/ijost.v6i2.34544 |
p- ISSN 2528-1410 e- ISSN 2527-8045 |