Original Article

Hypolipidemic activity of Ceciwis ethanol extract on wistar rats induced by high fat in vivo

Heru Nurcahyo[1]*, Aldi Budi Riyanta[1], Rizki Febriyanti[1], Heri Sutanto[2], Wiwin Herdwiani[3]

[1]Department of Pharmacy, Politeknik Harapan Bersana, Tegal, Indonesia. [2]Department of Physics, Universitas Diponegoro, Semarang, Indonesia. [3]Department of Pharmacy, Universitas Setia Budi, Surakarta, Indonesia.

Correspondence: Heru Nurcahyo, Department of Pharmacy, Politeknik Harapan Bersana, Tegal, Indonesia. herunurcahyo7770@gmail.com

ABSTRACT

In Indonesia, cruciferous cabbage is known as Ceciwis (Brassica oleracea L. var. capitata f. alba Alef), a vegetable that can be consumed as a diet from Tegal is one of the plants rich in flavonoids and phenol proposed has an antidyslipidemic ac-tivity. The purpose of this study is to see how Ceciwis affect the lipid profile of Wistar rats (Rattus norvegicus) fed a high-fat diet. Method: Rats divided into seven groups: the normal group, the negative group, positive group, and the treatment group with several doses of ethanol extract Brassica oleracea L. var. capitata f. alba Alef (EEB) 125, 250, 500, and 1000mg/KgBW. Each test group was given a high-fat diet of egg yolk 2 times a day 2 mL/200g (p.o), as well as PTU (Propylthiouracil) 0.01% during the 2-week induction period, then for 2 weeks the extract was dosed and took cholesterol measurements to determine the lipid profile with parameters of total cholesterol (TC), low density lipoprotein (LDL), and tryglyceride (TG). Results: EEB activity at a dose of 1000mg/KgBW significantly <0.05 decreased lipid profiles for TC, LDL, and TG almost equiva-lent to simvastatin positive control. Conclusion: EEB at a dose of 1000 mg/KW containing flavonoids and phenols significantly reduced the TC, LDL, and TG profiles.

Keywords: Ceciwis, Ethanol extract, Hypolipidemic, In vivo

Introduction

Hypolipidemic is a decrease in triglycerides and cholesterol with an increase in high density lipoproteins. These efforts are used to decrease the activity of dyslipidemia which can interfere with the metabolic activity of carbohydrates, proteins and fats that cause various diseases [1]. Dyslipidemia activities can cause the risk of diseases such as heart disease, diabetes to the risk of death [2, 3]. Endothelial dysfunction becomes the beginning of atherosclerosis plaque deposits due to increased LDL in the blood vessels [4]. So it needs to be addressed by consuming plant extracts that have been proven hypolipidemia activity.

Access this article online	
Website: www.japer.in	**E-ISSN:** 2249-3379

How to cite this article: Nurcahyo H, Riyanta AB, Febriyanti R, Sutanto H, Herdwiani W. Hypolipidemic activity of Ceciwis ethanol extract on wistar rats induced by high fat in vivo. J Adv Pharm Educ Res. 2023;13(1):100-4. https://doi.org/10.51847/enXiIQzXM1

Large side effects are the cause of the increased use of herbal plants. Herbal plants were chosen because of the great potential in the territory of Indonesia. In addition, side effects are relatively small and cheaper [5]. Various plant extracts have been tested for hypolipidemia activity for the treatment of dyslipidemia. Ethanol extract and fraction from the seeds of Cassia tora L. Able to decrease total cholesterol by 71,25%, increase HDL by 19,18% and lower triglycerides by 38,46% [6]. Moringa oleifera Lam methanol extract is known to reduce the risk of LDL, VLDL, triglycerides, atherogenic index and increase HDL [7, 8]. Aqueous extract of Capparis spinosa L. (CS) at a concentration of 20 mg/ kg body weight was able to significantly lower plasma cholesterol through observation for 4 days in normal and diabetic rats [9]. Great potential for developed herbal plant extracts to be an option for hypolipidemic activity.

Ceciwis "cruciferous cabbage" (Brassica oleracea L. var. capitata f. alba Alef) has the potential to have hypolipidemia activity. Ceciwis contains volatiles such as phenolic aldehydes, alcohols, and ketones [10]. Red cabbage (Brassica oleracea L.) contains anthocyanin dyestuffs [11, 12]. In addition, Ceciwis is also known to contain flavonoids and flavonols that have antioxidant activity

[13-16]. The content of anthocyanins, flavonoids and flavonols has hypolipidemic activity [17]. For this reason, it is necessary to investigate the hypolipidemic activity of ceciwis extract and see the extent of cholesterol reduction in rats induced with high fat. Hypolipidemic activity can be performed by inducing rats with high fat. Zhang *et al*., (2020) reported optimization of extraction of R [18]. Laevigatae fruit and hypolipidemia activity by inducing rats with it was obtained that R. laevigatae extract affected lipid metabolism and oxidative stress in a high-fat diet. Jensi, V. and Gopu (2018) investigated T. Arjuna extract with quercetin, gallocatechin, luteolin, terminoside a content against hypolipidemia activity in rats [19]. So that this induction method in rats can be carried out to investigate ceciwis extract (Brassica oleracea L. var. capitata f. alba Alef) against hypolipidemic activity.

Materials and Methods

Tools

Reflux extractor, Rotary vacuum evaporator (Rotavapor, Buchi®), analytical balance (Precisa®), measuring cup (pyrex), magsonde for oral administration of extracts and tools for the manufacture of liver histology preparations, namely: cutting board, scalpel knife, tweezers, tissue cassette, automatic processor machine, vacuum machine, blocking machine, freezer (-20°C).

Ingredients

Ethanol, Ceciwis (Brassica oleracea var. capitata f. alba alef), distilled water, quail eggs, PTU, biolabo reagent, physiological NaCl 0.9 percent.

Sample treatments

Ceciwis samples were collected from farmers in the Tegal Regency of Central Java, Indonesia. Preparation included wet sorting, washing, chopping, dry sorting, and chopping. The chopped ceciwis is then ready to be extracted using the reflux method.

Methods of extraction

Reflux extraction using 70% ethanol solvent and a sample: solvent ratio of 1:10, i.e. 100 grams of finely ground shallots in 1000 ml 70% ethanol. Reflux lasts for four to five hours with 70% alcohol solvent with a heating temperature of 50 degrees Celsius, then the liquid extract is evaporated with an evaporator at 50 degrees Celsius, and then transferred closer to a water bath at 50 degrees Celsius.

Test animal preparation

Male white rats (Rattus norvegicus) of the Wistar strain, aged 8–10 weeks, were used as the study's population. They were obtained from the Pharmacy of Politeknik Harapan Bersama Indonesia animal laboratory. The experimental animals were then acclimatized for seven days with conventional feed and aquadest drink, with observations made on their behavior and health conditions, and then weighed following the acclimatization period.

Research method

This research is a laboratory experimental study where the induction of wistar rats with high fat is exogenously and endogenously. Exogenously, a high fat diet feed with quail egg yolk as the main ingredient was used, while endogenously, PTU was used for 14 days. Hyperlipidemic conditions were created by monitoring blood lipid levels in wistar rats with levels ranging from 107 to 207 mg/dL. Furthermore, for the treatment and administration of wistar rats during a 14-day period, they were separated into 7 groups, each with 5 test animals:

a. The first group (K normal) was made up of normolipidemic rats fed a standard diet without action with a high-fat diet.
b. The second group (K-) was made up of hyperlipidemic rats fed a high-fat diet without drugs.
c. The third group (H1) was hyperlipidemic rats fed a high-fat diet and ethanol extract (*Brassica oleracea* var. capitata f. alba alef) 125mg/KgBW (EEB 125).
d. The fourth group (H2) was hyperlipidemic rats fed a high-fat diet and ethanol extract (*Brassica oleracea* var. capitata f. alba alef) 250mg/KgBW (EAB 250).
e. The fifth group (H3) was hyperlipidemic rats fed a high-fat diet and ethanol extract (*Brassica oleracea* var. capitata f. alba alef) 500 mg/KgBW (EAB 500).
f. The sixth group (H4) was hyperlipidemic rats fed a high-fat diet and ethanol extract (*Brassica oleracea* var. capitata f. alba alef) 1000 mg/KgBW (EAB 1000).

The seventh group (K+) was hyperlipidemic rats that were given a high-fat diet and given the hypolipidemic drug simvastatin at a dose of 1.8 mg/KgBW/day.

Investigating the lipid profile

On wistar rats, lipid profiles were examined by drawing blood through the caudalis vein with a scalpel, centrifuging the blood, and extracting the serum. The total cholesterol levels in the blood were assessed using the cholesterol oxidase peroxidase-4-aminophenazone (CHOD-PAP) method. The glycerol-3-phosphate oxidase, peroxidase, and chromogenic reaction with 4-amonophenazone (GPO-PAP) method was used to determine the amounts of triglycerides.

Research ethics

This research was conducted based on the ethics of animal research, where the ethics of research was obtained by the Semarang Health Polytechnic Ethics Commission with the number 0686/EA/KEPK/2022.

Results and Discussion

A small sample of ceciwis and a simple procedure wasused to conduct phytochemical screening to determine whether secondary metabolites are present, which are beneficial to the human beings [20]. The results of qualitative compound identification based on observations can be seen in **Table 1**.

Table 1. Identification of ceciwis ethanol extract secondary metabolite compounds

Phytochemical test	Reactor	Color	Result	Reference
Flavonoid	EtOH+Mg+HCl	Orange	+	Orange-purple
Phenols	Extract + $FeCl_3$	Black	+	Blue-Black

The test results showed that the ethanolic extract of ceciwis contains flavonoids and phenols. The results of a qualitative test were conducted on extracts thought to have the most flavonoids and phenols compounds. It can be seen by the color change that occurs from clear yellow to red-orange and blue-black for identification of phenols. The higher the content of flavonoid and phenols compounds, the darker the color produced [21].

Flavonoids identical with quercetin has activity in inhibiting the work of the lipase enzyme, which in the body oil can be hydrolyzed into fatty acids. Quercetin has activity in inhibiting the work of the lipase enzyme, which in the body oil can be hydrolyzed into fatty acids. Through beta oxidation, fatty acids can also be transformed into acetyl CoA, which is a precursor to cholesterol. The presence of quercetin in shallots inhibits the growth of precursors, which leads to an increase in blood cholesterol levels. An increase in free radicals [22] can damage nucleic acids, proteins, and lipid membranes, potentially leading to cancer and liver damage. Furthermore, quercetin can lower the activity of the enzyme acyl-CoA cholesterol acyltransferase (ACAT), which regulates cholesterol absorption in the intestine as well as lipoprotein synthesis in the liver [23]. Flavonoids can also increase the lipase enzyme's breakdown of lipids, allowing fatty acids, monoglycerides, and cholesterol to be absorbed by intestinal mucosal cells while lipids are expelled with feces, resulting in lower cholesterol and triglyceride levels [24, 25].

Hyperlipidemia is a disease burden impressively associated with obesity and characterized by the presence of extraordinary fats in the blood serum. The lipid profile in our blood is generally expressed by the presence of fats in the blood, such as cholesterol and triglycerides [26].

Normal Total cholesterol (TC) levels in rats with total cholesterol values of above 107-207 mg/dL were compared to baseline in rats with total cholesterol values of above 107-207 mg/dL [27], and the treatment was given with various doses on days 14 to 28 while the lipid profile level of the rats was observed.

In addition to the standard tests above, tests were also carried out with mice Wistar hypercholesterolemia. With treatment using egg yolk and PTU for two weeks Serum average cholesterol level has increased from 65 ± 7.023 mg/dL to 214.508 ± 2.002 mg/dL, which means that you have hypercholesterolemia.

Table 2. Profile lipid TC

Extract dose	Day 14	SD	Day 28	SD	Sig
EEB125	214,302	0,003	150,972	0,011	0,0001
EEB250	212,026	0,024	140,226	0,032	0,0001
EEB500	216,402	0,046	131,522	0,044	0,0001
EEB1000	215,305	0,032	113,15	0,034	0,0001

For the results of the Paired T-Test between the administration of high fat on day 14 and after administration of ethanol extract on day 28, sig was found to be 0.0001, which means that there is a relationship between the time of administration of ethanol extract with a decrease in cholesterol levels.

Table 3. Profile lipid Low-density lipoprotein (LDL)

Extract dose	Day 14	SD	Day 28	SD	Sig
EEB125	91,302	0,023	80,760	0,044	0,081
EEB250	102,026	0,028	79,730	0,030	0,055
EEB500	106,702	0,040	90,47	0,050	0,092
EEB1000	95,052	0,038	38,552	0,058	0,0001

For the results of the Paired T-Test between before and after giving the extract, the value of sig found 0.0001 for a dose of EEB 1000, that the dose has a relationship between the time of administration of the ethanol extract with a decrease in LDL levels, other than that dose does not provide a significant reduction.

Table 4. Profile lipid triglycerides (TG)

Extract dose	Day 14	SD	Day 28	SD	Sig
EEB125	214,022	0,011	155,722	0,054	0,0001
EEB250	194,082	0,024	125,298	0,035	0,0001
EEB500	218,004	0,020	142,294	0,042	0,0001
EEB1000	210,008	0,035	130,002	0,031	0,0001

For the results of the Paired T-Test between the administration of high fat on day 14 and after administration of ethanol extract on day 28, sig was found to be 0.0001, which means that there is a relationship between the time of administration of ethanol extract with a decrease in TG levels.

Giving EEB for 14 days showed a good downward trend in lipid profile levels (TC, LDL, and TG), indicating specific hypolipidemic activity. Data on lipid levels acquired on day 14 compared to a decrease in lipid levels on days 28, indicating specific hypolipidemic activity. The data were analyzed using a paired t test to compare the negative control with the treatment to determine lipid profile activity; the sign (*) with a significant value of p value is significant (0.05) where there is a significant decrease in (A) total cholesterol lowering activity, (B) LDL lowering activity, and (C) triglyceride lowering activity.

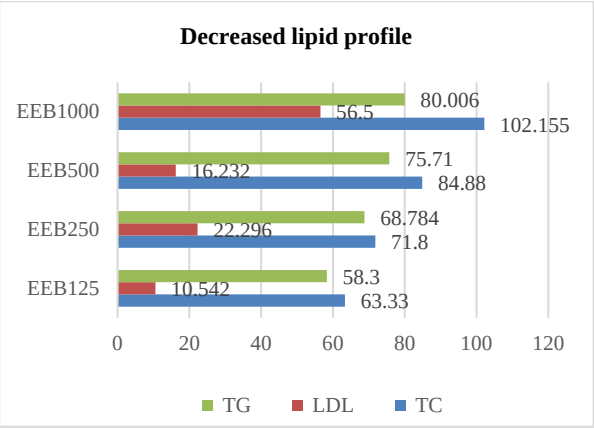

Figure 1. Decreased lipid profile

EEB 1000 extract dose had a significant decrease for TC, LDL and TG, where the compound Phenolics increase cholesterol metabolism [28]. Several phenolic compounds such as b-sitosterol have been proven to increase cholesterol metabolism. Phenolics are one of the antioxidant groups [29]. Antioxidants have hypolipidemic activity because they are able to activate LDL-cholesterol receptors so that LDL-cholesterol levels in plasma decrease [4, 19]. flavonoids are able to reduce LDL, increase LDL density through receptors in the liver and are able to bind to apolipoprotein B. Additionally, flavonoids can inhibit the work of the enzyme 3-hydroxy 3-methylglutaril coenzyme A reductase (HMG Co-A reductase) to reduce trigliseride and to increase HDL [2, 5, 12].

Conclusion

Based on the results of research and discussion, then it can be concluded that EEB activity at a dose of 1000mg/KgBW significantly <0.05 decreased lipid profiles for TC, LDL, and TG almost equivalent to simvastatin positive control. Conclusion: EEB at a dose of 1000 containing flavonoids and phenols significantly reduced the TC, LDL, and TG profiles

Acknowledgments: This research no potential conflict of interest was reported by the authors, We would like to thank Direktorat Riset dan Pengabdian Masyarakat (DRPM) from Ministry of Research and Technology.

Conflict of interest: The researcher stated that there was no conflict of interest in the manuscript and the code of ethics was violated and was in accordance with the provisions.

Financial support: We would like to thank Direktorat Riset dan Pengabdian Masyarakat (DRPM) from Ministry of Research and Technology, Higher Education for funding this research through the "PKPT grant scheme" program with number 6/065012/PPKM.PTV/SP2H/AK.04/022.

Ethics statement: The researcher stated that no code of ethics was violated and in accordance with the provisions.

References

1. Nugraheni K, Bintari SH. Aktivitas antidislipidemia Tepung tempe dan susu kedelai pada profil lipid tikus diabetes yang diinduksi streptozotocin. Indones J Nutr Diet. 2017;4(3):147-53. doi:10.21927/ijnd.2016
2. Manoharan K, Chitra P. Hypoglycemic and Hypolipidemic Activities of Ethanolic Extract of Elaeocarpus tectorius (Lour.) Poir. Leaves in Streptozotocin-Nicotinamide Induced Diabetic Rats. Biomed Pharmacol J. 2022;15(2):1167-78. doi:10.13005/bpj/2453
3. Soep S, Agussalim A. The impact of health education about Diabetes mellitus on patient knowledge to control their Blood Sugar. J Adv Pharm Educ Res. 2020;10(3):141-5.
4. Aprilia CA. Efektivitas Hipolipidemia dan Antioksidan Ekstrak Daun Binahong pada Tikus Putih yang Diinduksi Pakan Hiperkolesterol. J Kedokt YARSI. 2017;25(3):150-62. doi:10.33476/jky.v25i3.362
5. Ranti GC, Fatimawali F, Wehantouw F. Uji efektivitas ekstrak flavonoid dan steroid dari Gedi (abelmoschus manihot) sebagai anti obesitas dan Hipolipidemik pada tikus putih jantan galur wistar. Pharmacon. 2013;2(2):34-8.
6. Patil UK, Saraf S, Dixit VK. Hypolipidemic activity of seeds of Cassia tora Linn. J Ethnopharmacol. 2004;90(2-3):249-52. doi:10.1016/j.jep.2003.10.007
7. Jain PG, Patil SD, Haswani NG, Girase MV, Surana SJ. Hypolipidemic activity of Moringa oleifera Lam., Moringaceae, on high fat diet induced hyperlipidemia in albino rats. Rev Bras Farmacogn. 2010;20:969-73. doi:10.1590/S0102-695X2010005000038
8. Sinuraya RK, Rianti A, Suwantika AA. Cost minimization of cardiovascular disease (CVD) drugs in primary healthcare centers in Bandung, Indonesia. J Adv Pharma Educ Res. 2021;11(1):63-9. doi:10.51847/rzqepcg
9. Eddouks M, Lemhadri A, Michel JB. Hypolipidemic activity of aqueous extract of Capparis spinosa L. in normal and diabetic rats. J Ethnopharmacol. 2005;98(3):345-50. doi:10.1016/j.jep.2005.01.053
10. Protska V, Fedosov A, Burda N, Dobrovolnyi O, Dababneh MF, Kuznetsova M, et al. The study of volatile fractions of cabbage leaves (Brassica oleracea L. convar. capitata (L.) Alef. var. alba DC.) and determination of its antibacterial and antifungal activity. Int J Health Allied Sci. 2021;7(1):23-30.
11. Ahmadiani N, Robbins RJ, Collins TM, Giusti MM. Anthocyanins contents, profiles, and color characteristics of red cabbage extracts from different cultivars and maturity stages. J Agric Food Chem. 2014;62(30):7524-31. doi:10.1021/jf501991q
12. Pirozzi AV, Imbimbo P, D'Agostino A, Tirino V, Finamore R, Monti DM, et al. Antioxidant and hypolipidemic activity of açai fruit makes it a valuable functional food. Antioxidants. 2020;10(1):1-15. doi:10.3390/antiox10010040

13. Kusznierewicz B, Bartoszek A, Wolska L, Drzewiecki J, Gorinstein S, Namieśnik J. Partial characterization of white cabbages (Brassica oleracea var. capitata f. alba) from different regions by glucosinolates, bioactive compounds, total antioxidant activities and proteins. LWT-Food Sci Technol. 2008;41(1):1-9. doi:10.1016/j.lwt.2007.02.007
14. Hwang ES, Hong EY, Kim GH. Determination of bioactive compounds and anti-cancer effect from extracts of Korean cabbage and cabbage. Korean J Food Nutr. 2012;25(2):259-65.
15. Ciska E, Karamac M, Kosiñska A. Antioxidant activity of extracts of white cabbage and sauerkraut. Pol J Food Nutr Sci. 2005;14(4):367-73.
16. Mizgier P, Kucharska AZ, Sokół-Łętowska A, Kolniak-Ostek J, Kidoń M, Fecka I. Characterization of phenolic compounds and antioxidant and anti-inflammatory properties of red cabbage and purple carrot extracts. J Funct Foods. 2016;21:133-46. doi:10.1016/j.jff.2015.12.004
17. Jurgoński A, Juśkiewicz J, Zduńczyk Z. Ingestion of black chokeberry fruit extract leads to intestinal and systemic changes in a rat model of prediabetes and hyperlipidemia. Plant Foods Hum Nutr. 2008;63(4):176-82. doi:10.1007/s11130-008-0087-7
18. Zhang X, Hu Y, Jin C, Wu W. Extraction and hypolipidemic activity of low molecular weight polysaccharides isolated from Rosa Laevigata fruits. BioMed Res Int. 2020;2020. doi:10.1155/2020/2043785
19. Jensi VD, Gopu PA. Evaluation of Hypolipidemic Activity of Various Phytoconstituents from Terminalia arjuna (Roxb. ex DC.) in Rat Fed with High Fat Diet. Int J Pharm Sc Rev Res. 2018;50(2):41-8. doi:10.13140/RG.2.2.30541.49127
20. Hetta MH, Moawad AS, Hamed MA, Sabri AI. In-vitro and in-vivo hypolipidemic activity of spinach roots and flowers. Iran J Pharm Res. 2017;16(4):1509-19.
21. Nurcahyo H, Sumiwi SA, Halimah E, Wilar G. Total Flavonoid Levels of Ethanol Extract and Ethyl Acetate Fraction Dry Shallots (Allium cepa L. var. Garden Onion of Brebes) with Maceration Methods Using UV-Vis Spectrophotometry. Syst Rev Pharm. 2020;11(10):286-9. doi:10.31838/srp.2020.10.48
22. Saida K, Sara H, Amel B, Samira M. Free radical scavenging and biological activity of leaves and roots of Thymelaeahirsuta, collected from Algeria. Japer. 2020;10(4):15-20.
23. Deng W, Yang X, Zhu Y, Yu J, Xu X. Structural characterization and hypolipidemic activities of purified stigma maydis polysaccharides. Food Sci Nutr. 2019;7(8):2674-83. doi:10.1002/fsn3.1123
24. Ilyas M, Montana C, Malik F, Susanty S. Penurunan Kadar Trigliserida Tikus Putih Wistar Jantan (Rattus novergicus) Hiperlipidemik yang Diberi Ekstrak Terpuifikasi Batang Galing (Cayratia trifolis L. Domin.). Med Sains. 2020;4(2):79-86.
25. Kong F, Ding Z, Zhang K, Duan W, Qin Y, Su Z, Bi Y. Optimization of extraction flavonoids from Exocarpium Citri Grandis and evaluation its hypoglycemic and hypolipidemic activities. J Ethnopharmacol. 2020;262:113178. doi:10.1016/j.jep.2020.113178
26. Elsewedy HS, Shehata TM, Almostafa MM, Soliman WE. Hypolipidemic Activity of Olive Oil-Based Nanostructured Lipid Carrier Containing Atorvastatin. Nanomaterials. 2022;12(13):2160. doi:10.3390/nano12132160
27. Mohebbati R, Khajavi Rad A, Naser Shafei M, Soukhtanloo M, Hosseinian S, Beheshti F, et al. The Effects of Vitamin C on Adriamycin-Induced Hypercholesterolemia in Rat. Curr Nutr Food Sci. 2015;11(4):309-14. doi:10.2174/1573401311666150729230335
28. Tabet A, Boukhari A, Nouidjem Y. Phenolic content, HPLC analysis and antioxidant activity extract from Tamarix gallica and Tamarix articulata growing in southeast of Algeria. Res J Pharm Technol. 2018;11(9):3826-32. doi:10.5958/0974-360X.2018.00701.1
29. Sinha R, Varma R. Antioxidant activity in leaf extracts of Michelia champaca L. J Adv Pharm Educ Res. 2017;7(2):86-8.