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RESEARCH ARTICLE

Effectiveness of Lime Peel Extract (*Citrus aurantifolia* Swingle) against C-Reactive Protein Levels in Alloxan-Induced Wistar Rats

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Abstract

Hyperglycemia is a metabolic disease that is most often found and continuously increasing. Various complications due to hyperglycemia in the blood can cause tissue damage. It will increase free radicals that can trigger an inflammatory response characterized by an increased C-reactive protein in the blood. Prevention can be done by administering flavonoid antioxidant and lime peel containing high flavonoid. This study aims to analyze the efficacy of lime peel extract against C-reactive protein level with hyperglycemia through alloxan-induced Wistar rats (140 mg/kgBW). It is an experimental study using a post-test control group design that was carried out at the Pharmacology Laboratory of the Universitas Surabaya for the period July–August 2020. Experimental Wistar rats were divided into a negative control group, a positive control group, and three groups with different doses of lime peel extract (2.35 mg, 4.7 mg, and 9.4 mg). Treatment was carried out for 30 days before measuring the C-reactive protein levels in the blood using ELISA. The results showed a difference in C-reactive protein level between groups (Man-Whitney, p=0.004). The increase in the dose of lime peel extract (9.4 mg) showed the lowest C-reactive protein level. Therefore, it can be concluded that the administration of lime peel extract in hyperglycemia conditions can reduce the inflammatory process in the body.

Key words: Alloxan, C-reactive protein, hyperglycemia, inflammation, lime

Efek Ekstrak Kulit Jeruk Nipis (*Citrus aurantifolia* Swingle) terhadap Kadar *C-Reactive Protein* pada Tikus Wistar yang Diinduksi Aloksan

Abstrak

Hiperglikemia merupakan penyakit metabolik yang paling sering dijumpai dan terus mengalami peningkatan dari tahun ke tahun. Berbagai komplikasi akibat hiperglikemia dalam darah dapat menyebabkan kerusakan jaringan. Hal ini dikarenakan hiperglikemia akan meningkatkan radikal bebas sehingga memicu respons inflamasi yang ditandai dengan peningkatan *C-reactive protein* dalam darah. Pencegahan dapat dilakukan dengan pemberian asupan antioksidan flavonoid. Kulit jeruk nipis memiliki kandungan flavonoid yang tinggi. Penelitian ini bertujuan menganalisis efikasi ekstrak kulit jeruk nipis terhadap kadar *C-reactive protein* pada tikus Wistar dengan kondisi hiperglikemia melalui induksi aloksan (140 mg/kgBB). Metode pada penelitian ini adalah eksperimental dengan menggunakan *post-test control group* yang dilaksanakan di Laboratorium Farmakologi Universitas Surabaya periode Juli–Agustus 2020. Hewan coba tikus Wistar dibagi menjadi kelompok kontrol negatif, kelompok kontrol positif, dan tiga kelompok perlakuan dengan pemberian dosis ekstrak jeruk nipis yang berbeda (2,35 mg; 4,7 mg; dan 9,4 mg). Pemberian perlakuan dilakukan selama 30 hari, selanjutnya akan dilakukan pengukuran kadar *C-reactive protein* dalam darah dengan menggunakan ELISA. Hasil penelitian memperlihatkan perbedaan kadar *C-reactive protein* antarkelompok (Mann-Whitney, p=0,004). Peningkatan pemberian dosis ekstrak kulit jeruk nipis (9,4 mg) menunjukkan penurunan kadar *C-reactive protein* paling rendah. Oleh karena itu, dapat disimpulkan bahwa pemberian ekstrak kulit jeruk nipis pada kondisi hiperglikemia dapat menurunkan proses inflamasi dalam tubuh.

Kata kunci: Aloksan, *C-reactive protein*, hiperglikemia, inflamasi, jeruk nipis

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Introduction

Hyperglycemia is a condition where glucose levels in the blood increased due to insulin resistance, resulting in an imbalance of insulin concentration and plasma glucose levels.[1] Persisting hyperglycemia will lead to chronic diseases such as type 2 diabetes caused by decreased β cell function and insulin secretion in the blood.[2] Diabetes mellitus is classified as a non-communicable disease that requires special attention. Non-communicable diseases have increased each year significantly, estimated to have contributed 72% of all deaths due to disease in 2016.[3] The increase in people with diabetes mellitus has also increased to reach 425 million people in 2017 with around 4 million deaths. It is expected to reach 629 million people in 2045.[4] Besides, diabetes also causes disabilities and complications, mainly in the heart and kidneys.[5]

Increased blood glucose in people with diabetes will stimulate the production of excessive free radicals in the body. Various free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) will be produced and cause oxidative stress.[6,7] This happens to the increasing amount of excessive free radicals that result in an imbalance in the number of antioxidants present in the body.[8] Oxidative stress will cause direct damage to cell tissue through the lipid peroxidase reaction.[9] Cell tissue damage caused by an excessive increase of free radicals is often referred to as cell debris or damage associated molecular patterns (DAMPs).[10] Increasing the number of debris cells in the body's microenvironment will stimulate macrophages' movement as one of the body's primary defense systems for phagocytosis. Debris cells that macrophages have phagocyted will trigger inflammatory reaction through the secretion of pro-inflammatory cytokines such as interleukin-1, interleukin-6, interleukin-8, and tumor necrosis factor alpha (TNF-α).[11]

The impact of an inflammatory reaction caused by hyperglycemia is an increased risk of atherosclerotic diseases such as heart disease and stroke. Increased pro-inflammatory cytokines such as interleukin-1, interleukin-6, and TNF-α will induce the liver to release the acute phase of the protein C-reactive protein.[12] Increased levels of C-reactive protein in the blood are not only a sign of inflammation in the body, but C-reactive protein also plays an essential role in the inflammatory response, which can further accelerate cell damage.[13] Increased free radicals and inflammatory responses can be prevented by administering antioxidant intake from the outside. The number of antioxidants that the body needs to neutralize free radicals is sufficient, then the lipid peroxidase reaction that can damage the tissue can be reduced.[14]

Flavonoids are antioxidants that can be found in fruits, especially lime (*Citrus aurantifolia* Swingle). In recent years, research on the use of flavonoids as antioxidants in food sources is increasing. It has an impact on the increasing number of processed products from fruit. Each treatment process can reduce the flavonoid content by up to 50%.[15] The considerable amount of flavonoids on lime peel can be an alternative source of antioxidants.

This study used lime peel as a source of antioxidant flavonoids, so it is expected to increase antioxidant levels in the body. The intake of lime peel extract was carried out through the extraction process and initial testing of experimental Wistar rats (*Rattus norvegicus*) induced by alloxan.

Methods

This research was an experimental study using a post-test control group design. The research process was carried out on male Wistar rats (*Rattus norvegicus*) as experimental animals for 30 days and has passed the ethical test at the Institutional Ethical Committee, University of Surabaya (No.: 137/KE/VI/2020). Experimental Wistar rats will be divided into negative control groups, positive control groups, and three groups with different doses of lime peel extract.

This study used Wistar rats as an experimental animal. Some requirements are given to animals to make them homogeneous. They are 2–3 months old, weighing ±200 grams, have no macroscopic abnormalities, and have never been used for the object of research. The study was conducted at the Pharmacology Laboratory of the Faculty of Medicine, Universitas Surabaya. Each treatment group will be given a lime peel extract with different doses of 2.35 mg, 4.7 mg, and 9.4 mg.

Lime peel (about 2 kilograms) was cleaned and dried. Furthermore, powder preparations will be made using a blender and carried out

sifting to obtain fine and homogeneous powder (±40 mesh). The next step was extraction using the maceration method with 96% ethanol solvent (±10 liters). Leave for 3×24 hours, and every 24 hours, change the solvent until the resulting filtrate is clear. Concentration results will obtain dense preparation using a vacuum rotary evaporator.[16] Then, the flavonoid level in lime peel extract will be measured (51.23 mg/g), and a conversion table between organisms Laurence and Bacharach will be calculated so that doses of 2.35 mg, 4.7 mg, and 9.4 mg are obtained.[17]

After the adaptation process for 5–7 days, the experimental rats fasted for 6–8 hours. Then they will be given a single alloxan injection of 140 mg/kgBW (diluted with NaCl 0.9%) intraperitoneally. Experimental animals received blood glucose tests before the research began, so the positive control group and the treatment group reached a hyperglycemia condition. Hyperglycemia condition in experimental animals was compared with other treatment groups (mean 89.00±4.00, p=0.519), then with the negative groups (mean 75.4±3.78, p=0.001).

Measurement of C-reactive protein level in the blood serum is done by using ELISA. The result is indicated positive when agglutination is present, and C-reactive protein level reached a level of ≥6 mg/L. The result is indicated negative when there is no agglutination. The level of C-reactive protein is below 6 mg/L.[18] The C-reactive protein level can increase significantly above the normal level with the onset of substantial inflammatory stimulus.

This research was conducted for 30 days by dividing up to 5 treatment groups. The first group is a negative control group where the experimental animals were not given any treatment for 30 days. In the second to the fifth group, alloxan was administered to increase the blood glucose level to achieve hyperglycemia. The second group is a positive control group used to compare where experimental animals that have reached the condition of hyperglycemia are given glimepiride 0.36 mg (equivalent to 2 mg/day in adults). Glimepiride was used in this study because it is effective in reducing blood glucose levels.[19] For the other three treatment groups were also hyperglycemia conditioned and were given extracts of lime peel at a dose of 2.35 mg, 4.7 mg, and 9.4 mg.

The result of the study will obtain ordinal data in the form of C-reactive protein level in each group in mg/L units. C-reactive protein assessment level of >6 mg/L, 12 mg/L and 24 mg/L will be given coding (non-parametric). Data analysis was performed by using Kruskal-Wallis analysis with SPSS version 22 to see differences between groups. The difference between the two groups was significant when the p-value<0.05.

Results

The results of this study were carried out by comparing C-reactive protein levels between groups. Table 1 shows the results of measurement of C-reactive protein level in blood serum between groups. In groups, I and V, C-reactive protein levels in all experimental animals showed the lowest value <6 mg/L, while the highest C-reactive protein level was found in group II.

Table 2 shows the results of the Kruskal-Wallis test on C-reactive protein level between groups were 0.004 (p-value<0.05), so it can be concluded that there are significant differences in C-reactive protein level.

Research data on C-reactive protein levels in each group were also analyzed to show how much

Table 1 C-Reactive Protein Level in Experimental Animal

Groups	Negative Control	Positive Control	Treatment I	Treatment II	Treatment III
C-reactive protein level (mg/L)	<6	24	<6	<6	<6
	<6	24	<6	<6	<6
	<6	12	12	<6	<6
	<6	12	12	12	<6
	<6	12	12	12	<6

Table 2 Kruskal-Wallis Test Results on C-Reactive Protein Level between Groups

Groups	Kruskal-Wallis Test
Negative control	
Positive control	
Treatment I	0.004
Treatment II	
Treatment III	

Table 3 Mann-Whitney Test Result C-Reactive Protein Level between Groups

Groups	I	II	III	IV	V
I	–	–	–	–	–
II	0.008	–	–	–	–
III	0.032	0.032	–	–	–
IV	1.000	0.008	0.032	–	–
V	1.000	0.008	0.032	1.000	–

influence the lime peel extract has in reducing C-reactive protein levels than the negative and positive control groups. Based on Table 3, shows the differences in each group using the Mann-Whitney test.

The results show that groups I, IV, and V have the same C-reactive protein value (p value=1.000). In comparison, groups II and III showed significant differences with group I (p value<0.05).

Discussion

The negative control group did not show an increase in C-reactive protein levels. In contrast, the positive control group showed an increase in C-reactive protein levels, although antidiabetic drugs were administered. It can be concluded that in hyperglycemia conditions that have been given antidiabetic drugs, there is still an inflammatory reaction.

Hyperglycemia causes damage to endothelial cells, which can stimulate free radical formation.[20] Normally, free radicals can be neutralized by antioxidants in the body or often called enzymatic antioxidants.[21] Various types of enzymatic antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) are types of antioxidants that can not be given through intake from outside the body yet play a significant role in neutralizing free radicals.[22] Antioxidant superoxide dismutase (SOD) can neutralize superoxide radicals ($O_2^{\bullet -}$) into hydrogen peroxidase (H_2O_2), while antioxidant glutathione peroxidase (GSH-Px) and catalase (CAT) functioning to change hydrogen peroxidase into non-radical formations that are water (H_2O) and oxygen (O_2).[23] Therefore giving antioxidant intake can be done by consuming non-enzymatic antioxidants such as flavonoids.[24]

Giving antioxidants using lime peel extract can reduce levels of C-reactive protein in the blood. The treatment group showed that the greater the dose of lime peel extract, the lower the blood's C-reactive protein level. It shows that the flavonoid content in lime peel extract can reduce free radicals and prevent an increase in the inflammatory response.

Most of the flavonoids can act as antioxidants. Flavones and catechins are the strongest flavonoids to protect the body from free radicals. Flavonoids will be oxidized by free radicals (such as superoxides and peroxynitrite) and produce more stable and less reactive radicals. Free radicals will decrease the ability to cause cell damage.[25]

This study is expected to be the first step in considering hyperglycemia conditions because anti-diabetes therapy has not fully reduced the negative impact of free radicals and will trigger an inflammatory response. The development of lime peel extract can be an alternative to reduce inflammation in hyperglycemic conditions.

Conclusions

Giving blood glucose-lowering drugs in the form of glimepiride in hyperglycemia conditions does not respond to decreased inflammation. However, the administration of lime peel extract (9.4 mg) can reduce the inflammatory reaction in the body.

Conflict of Interest

The authors declare none.

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References

1. Plummer MP, Finnis ME, Phillips LK, Kar P, Bihari S, Biradar V, et al. Stress induced hyperglycemia and the subsequent risk of type 2 diabetes in survivors of critical illness. PLoS One. 2016;11(11):e0165923.
2. Lee PG, Halter JB. The pathophysiology of hyperglycemia in older adults: clinical considerations. Diabetes Care. 2017;40(4):444–52.
3. GBD 2016 Causes of Death Collaborators. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet. 2017;390(10100):1151–210.
4. Tripathy JP. Burden and risk factors of diabetes and hyperglycemia in India: findings from the Global Burden of Disease Study 2016. Diabetes Metab Syndr Obes. 2018;11:381–7.
5. Stanifer JW, Cleland CR, Makuka GJ, Egger JR, Maro V, Maro H, et al. Prevalence, risk factors, and complications of diabetes in the Kilimanjaro region: a population-based study from Tanzania. PLoS One.2016;11(10):e0164428.
6. Matough FA, Budin SB, Hamid ZA, Alwahaibi N, Mohamed J. The role of oxidative stress and antioxidants in diabetic complications. Sultan Qaboos Univ Med J. 2012;12(1):5–18.
7. Asmat U, Abad K, Ismail K. Diabetes mellitus and oxidative stress—a concise review. Saudi Pharm J. 2016;24(5):547–53.
8. Suryadinata RV, Wirjatmadi B, Adriani M. Efektivitas penurunan malondialdehyde dengan kombinasi suplemen antioksidan superoxide dismutase melon dan gliadin akibat paparan rokok. GMHC. 2017;5(2):79–83.
9. Suryadinata RV, Adriani M, Martini S, Sumarmi S, Wirjatmadi B. The role of selenium micronutrients as antioxidants in exposure to e-cigarette smoke. Asian J Pharm Clin Res. 2019;12(8):265–8.
10. Vénéreau E, Ceriotti C, Bianchi ME. DAMPs from cell death to new life. Front Immunol. 2015;6:422.
11. Suryadinata RV. Pengaruh radikal bebas terhadap proses inflamasi pada penyakit paru obstruktif kronis (PPOK). Amerta Nutr. 2018;2(4):317–24.
12. Boras E, Slevin M, Alexander MY, Aljohi A, Gilmore W, Ashworth J, et al. Monomeric C-reactive protein and Notch-3 co-operatively increase angiogenesis through PI3K signalling pathway. Cytokine. 2014;69(2):165–79.
13. Sproston NR, Ashworth JJ. Role of C-reactive protein at sites of inflammation and infection. Front Immunol. 2018;9:754.
14. Suryadinata RV, Wirjatmadi B, Adriani M. Pengaruh perubahan hiperplasia sel goblet selama 28 hari paparan asap rokok dengan pemberian antioksidan superoxide dismutase. IJPH. 2017;11(1):60–8.
15. Gattuso G, Barreca D, Gargiulli C, Leuzzi U, Caristi C. Flavonoid composition of citrus juices. Molecules. 2007;12(8):1641–73.
16. Assagaf M, Hastuti P, Hidayat C, Supriyadi. Optimasi ekstraksi oleoresin pala (*Myristica fragrans* Houtt) asal Maluku Utara menggunakan response surface methodology (RSM). Agritech. 2012;32(4):383–91.
17. Suryadinata RV, Wirjatmadi B. Selenium linked to increased antioxidant levels and decreased free radicals in lung tissue of Wistar rats exposed to e-cigarette smoke. JGPT. 2020;12(9):32–9.
18. Kalma. Studi kadar C-reactive protein (CRP) pada penderita diabetes melitus tipe 2. J Media Analis Kes. 2018;1(1):62–8.
19. Basit A, Riaz M, Fawwad A. Glimepiride: evidence-based facts, trends, and observations. Vasc Health Risk Manag. 2012;8:463–72.
20. Tanaka M, Masuda S, Matsuo Y, Sasaki Y, Yamakage H, Muranaka K, et al. Hyperglycemia and inflammatory property of circulating monocytes are associated with inflammatory property of carotid plaques in patients undergoing carotid endarterectomy. J Atheroscler Thromb. 2016;23(10):1212–21.
21. Suryadinata RV, Wirjatmadi B, Adriani M, Sumarmi S. The effects of exposure duration to electronic cigarette smoke on differences in superoxide dismutase and malondialdehyde in blood of Wistar rats. Int J Curr Pharm Res.

2019;11(3):13–6.
22. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem. 2015;30(1):11–26.
23. Petersen RC, Reddy MS, Liu PR. Advancements in free-radical pathologies and an important treatment solution with a free-radical inhibitor. SF J Biotechnol Biomed Eng. 2018;1(1):1003.
24. Pratiwi SR, Lorensia A, Suryadinata RV. Asupan vitamin C dan E dengan SQ-FFQ terhadap fungsi paru perokok dan non-perokok. MKMI. 2018;14(2):101–7.
25. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci. 2016;5:e47.