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 Alokson

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 respon 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: Alokson, 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. Persisting hyperglycemia will lead to chronic diseases such as type 2 diabetes caused by decreased β cell function and insulin secretion in the blood. 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. 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.

Besides, diabetes also causes disabilities and complications, mainly in the heart and kidneys. 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. This happens to the increasing amount of excessive free radicals that result in an imbalance in the number of antioxidants present in the body. Oxidative stress will cause direct damage to cell tissue through the lipid peroxidase reaction. Cell tissue damage caused by an excessive increase of free radicals is often referred to as cell debris or damage associated molecular patterns (DAMPs).

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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, interleukin-8, and tumor necrosis factor alpha (TNF-α) will induce the liver to release the acute phase of the protein C-reactive protein. 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. 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.

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%. 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. 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.

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. 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. 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

| 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 |
Antioxidant intake can be done by consuming non-enzymatic antioxidants such as flavonoids. 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.

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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