

Reduction of free radicals in hyperglycemic conditions through the administration of lime peel extract (*Citrus aurantifolia* swingle)

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ABSTRAK

Latar Belakang: Kondisi hiperglikemia tidak selalu terjadi pada penderita diabetes. Stress oksidatif merupakan salah satu proses pathogenesis yang ditimbulkan terhadap penyakit akibat hiperglikemia. Hal tersebut berkaitan dengan peningkatan Reactive Oxygen Species (ROS) dan penurunan aktivitas antioksidan. Pemberian asupan antioksidan dari luar tubuh diharapkan dapat membantu menetralkan radikal bebas yang berlebihan.

Tujuan: Untuk mengetahui efektivitas ekstrak kulit jeruk nipis terhadap penurunan radikal bebas pada kondisi hiperglikemia.

Metode: Penelitian ini merupakan eksperimental dengan post-test control group design dengan menggunakan tikus wistar di laboratorium Fakultas Kedokteran, Universitas Surabaya. Kondisi hiperglikemia pada hewan coba dilakukan melalui induksi aloksan dan selanjutnya diberikan ekstrak kulit jeruk nipis (2,35 mg; 4,7 mg; 9,4 mg) selama 30 hari dengan membagi menjadi 5 kelompok perlakuan. Parameter untuk mengetahui kadar radikal bebas pada penelitian ini adalah kadar malondialdehid

Hasil: Pada penelitian memperlihatkan bahwa pada kelompok yang diberikan ekstrak kulit jeruk nipis akan mengalami penurunan kadar malondialdehid jika dibandingkan dengan kelompok lainnya ($p < 0,05$). Hasil rerata kadar malondialdehid terendah pada dosis 9,4 mg ($1,67 \pm 0,10$)

Kesimpulan: Pemberian ekstrak kulit jeruk nipis dapat menurunkan kadar malondialdehid pada kondisi hiperglikemia.

KATA KUNCI: *Citrus aurantifolia*; hiperglikemia; malondialdehid; radikal bebas

ABSTRACT

Background: Hyperglycemia condition does not always occur in diabetes. Oxidative stress is a process of pathogenesis diseases as a result of hyperglycemia. This is associated with increased Reactive Oxygen Species (ROS) and decreased antioxidant activity. Antioxidants intakes outside the body is expected to neutralize excessive free radicals.

Objectives: To determine the effectiveness of lime peel extract against free radicals induced by hyperglycemia.

Methods: This research is an experimental post-test control group design using wistar rats in the laboratory of the Faculty of Medicine, University of Surabaya. Hyperglycemia conditions in experimental animals were carried out through alloxan induction and then given lime peel extract (2,35 mg; 4,7 mg; 9,4 mg) for 30 days by dividing into 5 treatment groups. The parameter to determine the levels of free radicals in this study is the levels of malondialdehyde

Results: Showed that the group given lime peel extract experienced a decrease in malondialdehyde levels when compared to the other groups ($p < 0.05$). The lowest mean malondialdehyde level was at a dose of 9.4 mg (1.67 ± 0.10).

Conclusion: The administration of lime peel extract can reduce levels of Malondialdehyde induced by hyperglycemia.

KEYWORDS: *Citrus aurantifolia*; free radicals; hyperglycemia; malondialdehyde

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INTRODUCTION

Increased blood glucose level, known as hyperglycemia, is commonly associated with diabetes due to increased glucose production and decreased absorption, rather than the usage of glucose as cellular fuel under stimulation of insulin. (1) Various risk factors, allied to life style changes have the potentials to affect blood glucose level, these include smoking, obesity, sedentary life, excessive carbohydrate intake, and hypercholesterolemia. Lack of ideal lifestyles aforementioned increase the risk of diabetes, i.e. type 2 diabetes. (2-5)

The formation of free radicals through glycation of non enzymatic proteins, glucose oxidations and increased lipid peroxidations often occurs in the hyperglycemic state. The unhealthy lifestyles may increase the levels of free radicals which the available antioxidant is not adequate to neutralize them. (6) This conditions will result enzyme damages and increased insulin resistance. (7) Chronic increased insulin resistance and oxidative stress reactions will further increase cellular damages. (8) Pathologically, both conditions will result in complications such as rheumatoid arthritis, diabetes mellitus, and cancer. While these that occur to people with diabetes might lead to coronary artery disease, diabetic nephropathy and retinopathy more common complications. (9)

Free radicals are active derivate of oxygen molecules such as Reactive Oxygen Species (Hydroperoxyl, Superoxide, Hydrogen Peroxide and Hydroxyl Radicals) and niotrogen molecule (Reactive Nitrogen Species, i.e. Peroxynitrite). Free radicals in their nature are highly reactive due to its absence of paired molecules in the outer molecule. (10) In turn, they will trigger lipid oxidation reaction, protein and DNA, which will possibly make cell damage. (11) While the majority of biological cells have an intrinsic defensive mechanism involving enzymes such as superoxide dismutase (SOD), catalase and glutation peroksidase (GSH-Px), but the amount is not balanced. (12) The secretion of insulin plays a role in reducing the level of glucose in the blood and its metabolism. Impaired secretory function or what is often referred to as insulin

resistance will result in blood glucose cannot be used in cell metabolism. This decrease in sensitivity will result in an excessive increase in insulin secretion, resulting in saturation and triggering type 2 diabetes. (13)

Free fatty acids in plasma will increase to be used as the main source of metabolism. However, chronic increases in free fatty acids will cause insulin disorders and the accumulation of fat metabolites in the liver and muscles such as fatty acyl-coenzyme A and diacylglycerol. Increased levels of free fatty acids in the blood will trigger an increase in the production of free radicals in cells. (14) This is because mitochondria as a producer of free radicals have increased substrate production. The accumulation of fat metabolites will also trigger the inflammatory process and the production of free radicals. Oxidative stress caused by free radicals in diabetics showed a significant increase compared to normal people. Increased free radicals in people with type 2 diabetes are found in the blood, tissue and skeletal muscle. (15) The negative effect of free radicals can be ameliorated through by antioxidant intake from external sources i.e. non-enzymatic antioxidants. (16) Numerous types of antioxidants can be obtained from fruits which contain flavonoid and vitamins. (17)

Lime (*Citrus aurantifolia Swingle*) is one of food supplements with large number of flavonoids. Which are obtained not only from its main part (the pulp) but also its peel. (18) Therefore, this study aimed further understand the effect of antioxidant in lime peel to decrease free radicals induced by hyperglycemia. In initial form, this study used experimental animals, i.e. alloxan-induced white mice (*Rattus novergicus*).

MATERIAL AND METHODS

The study employed an experimental, post-test control group design. Male wistar mice (*Rattus novergicus*) were under experiment for the 30 days study and were cathegorized in 5 groups, negative and positive control and three experimental groups. Male wistar mice (*Rattus novergicus*) had to have criteria. First, they had approximate weight of ± 200 g and no macroscopic anomalies. They also should

never been included in any previous studies. Mice which were sick or dead during the experiment were excluded. The principle of 3R (*Replacement, Reduction, and Refinement*) to ensure the mice be in a good condition. (19)

Lime peel extract (*Citrus aurantifolia* Swingle) was cleaned and dried, then blended into a powdery substance. Next, it was shaken to produce homogenous and soft powder. Further extraction was done using maceration method with a diluting agent of 96% ethanol. The substance as a result was kept for 3x24 hours, and every 24 hours its diluting agent was changed to keep the filtrate clean. The result was a thick substance extracted by a vacuum rotary evaporator. The level of flavonoids in the lime extract was measured in an Laurence and Bacharach conversion table calculations. The extracts obtained were 2.35 mg, 4.7 mg, and 9.4 mg, respectively.

Animals involved fasted for 6-8 hours were injected 140 mg/kg alloxan per body weight intraperitoneally (diluted with 0.9% NaCl). Before the research initiation, blood glucose levels of the animals were tested for positive control and experimental groups to achieve hyperglycemia. The research has received ethical clearance at the ethical committee University of Surabaya (No: 137/KE/VI/2020).

This study was conducted to 5 research groups in 30 days. The first group was a negative control group, where the animals were given daily doses without any interventions. The second group was positive control groups, where the experimental animals were only induced with alloxan and given daily routine intake (Pellet). Meanwhile, the other three groups were induced with alloxan, given daily routine intake and lime extract at different doses of 2.35 mg, 4.7 mg, and 9.4 mg, respectively.

The results were in ratios of malondialdehyde levels in each group. Data analysis was performed using the ANOVA test in SPSS version 22 to study the differences between groups. (20)

RESULTS AND DISCUSSION

The results obtained were compared between groups. **Table 1** shows the mean of malondialdehyde levels between control groups and experimental

ones. The obtained data have passed the normality test between groups ($p > 0.05$) and homogeneity test ($p = 0.232$) as a precondition for the ANOVA test. Table 1 presents that the ANOVA test approximately resulted in a significance level of 0.000 ($p < 0.05$).

Table 1. Means of malondialdehyde level between groups

	Group	Means \pm SD	P Values
I	Control Groups with intake, without aloksan induction and lime peel extract	1.80 \pm 0.12	
II	Control Groups with intake, with aloksan induction, without lime peel extract	2.16 \pm 0.12	
III	Control Groups with intake, with aloksan induction, with 2,35 mg of lime peel extract	1.89 \pm 0.09	0.000
IV	Control Groups with intake, with aloksan induction, with 4,7 mg of lime peel extract	1.68 \pm 0.04	
V	Control Groups with routine, with aloksan induction, with 9,4 mg of lime peel extract	1.67 \pm 0.10	

The basic mechanism of chronic hyperglycemia pertains to diabetes and complications as ones of its manifestations. Excessive nutritional intake will cause disruption of insulin resistance and secretion gradually so that glucose in the blood increases. The aforementioned effect happens in steps and are associated with other factors—aging, obesity, and decreased physical activities.(21) Persistent hyperglycemia induces toxic effects in either macrovascular and microvascular ones known as glucotoxicity. Oxidative stress affects the pathogenesis of glucotoxicity during the continuous diabetes and its complications.(22)

The increase in free radicals which is characterized by the occurrence of oxidative stress has been considered as one of the triggers for hyperglycemia. This will interfere with insulin retention, glucose tolerance to cell dysfunction. In pre-diabetic conditions, visceral fat and adipose tissue have expressed various pro-inflammatory cytokines such as

Tumor Necrosis Factor- α (TNF- α) and interleukin 6 (IL-6) which trigger inflammation and oxidative stress.(23) Diabetics are not only affected by diabetes. increased free radicals, but also decreased antioxidant activity. Therefore, the condition of hyperglycemia is considered to increase the risk of complications in diabetes. (24) Hyperglycemia has several mechanisms in increasing free radicals in DM patients. Several mechanisms that can increase free radicals are polyol (sorbitol) pathway flux, advanced glycation end-product (AGE) formation, activation of protein kinase C (PKC) isoforms, and hexosamine pathway flux. In addition, there is an endoplasmic reticulum which also determines the increase in free radicals. (25)

Malondialdehyde levels in hyperglycemia cannot decline or even be similar to those with normal blood sugar level as a result of superoxide produced by hyperglycemia for inhibiting and damaging the mitochondrial activity. The pathway involving oxidative stress is the activity that is inhibited by glycerol dehyde-3 phosphate dehydrogenase due to reactive oxygen species that have inhibited glyceraldehydes 3 phosphate dehydrogenase through a mechanism involving the poly-ADP ribose polymerase-1 enzyme. Stress oxidative as a result of hyperglycemia will increase stress signalling in beta cells that can stimulate apoptosis.(26)

The ANOVA test of malondialdehyde levels using the least significance difference (LSD) aimed to examine the differences between groups. Referring to Table 2, this study figures out that almost all groups had significant differences ($p < 0.05$). Insignificant differences were only found in the comparison between two groups, i.e., the negative and the first experiment group, as well as the second and third groups ($p > 0.05$).

The results showed from the experimental animals with hyperglycemia had increased free radicals. The increase was directly shown from higher blood levels as a products of lipid peroxide of the cellular membrane. The common use of anti-hyperglycemia medication merely decreases the blood glucose level, while the free radical levels remain high. Therefore, it is essential to use antioxidants as an external supplement to ameliorate

Table 2. Least Significance Differences (LSD) between groups

Groups	I	II	III	IV	V
I	-	-	-	-	-
II	0.000	-	-	-	-
III	0.111	0.000	-	-	-
IV	0.043	0.000	0.001	-	-
V	0.037	0.000	0.001	0.944	-

Description:

Groups I : Control Groups with intake, without aloksan induction and lime peel extract

Groups II : Control Groups with intake, with aloksan induction, without lime peel extract

Groups III : Control Groups with intake, with aloksan induction, with 2,35 mg of lime peel extract

Groups IV : Control Groups with intake, with aloksan induction, with 4,7 mg of lime peel extract

Groups V : Control Groups with routine, with aloksan induction, with 9,4 mg of lime peel extract

the effects of tissue injuries.(9) Supplementation of lime peel extract at certain doses has been proven to decrease the free radical levels induced by hyperglycemic. Flavonoids contained in the lime peel can effectively decrease the free radicals when a daily dose of extracts is administered.(27)

This study has limitations, namely researchers do not yet know all the antioxidant content found in lime peels

CONCLUSION AND RECOMMENDATIONS

Hyperglycaemia increased free radicals even though the anti-hyperglycaemic medication has been administered. However, this study concludes that the administration of lime peel extract as an external antioxidant can be effective to decrease free radicals induced by hyperglycemia. Decreased malondialdehyde levels are commonly used indicators to identify its effectivity. In future research, it is expected to find out the content of other antioxidants in lime peel which are useful for diabetics

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CONFLICT OF INTEREST

Declare none

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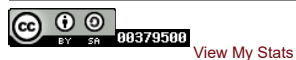
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[The addition of snakehead fish \(Channa Striata\) and bitter ginger \(Zingiber zerumbet L\) to fish floss](#)

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