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Effects of Giving Kaffir Kaffir Extract (*Citrus hystrix*) on Blood Glucose Levels in Wistar Rats

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ABSTRACT

Dyslipidemia is associated with cardiovascular disease as well as hyperglycemia and insulin resistance. One of the natural ingredients for contro[10]g dyslipidemia is kaffir lime peel (*Citrus hystrix*), which contains flavonoids and [23]olyphenols that act as antioxidants and regulate blood glucose. This study aims to prove the effect of kaffir lime peel in l[4]ering blood glucose levels. *Post-test only controlled group design study* , using 25 wistar rats divided into 5 groups, namely group (K-) without high-fat diet and extra[3] (K+) was given a high-fat diet alone, (P1) was given kaffir lime p[32]extract at a dose of 35 mg/kg BW rats/day, (P2) was [10]en extract at a dose of 70 mg/kg BW rats/day and (P3) was given extract at a dose of 140 mg. /kgBW rats/day. The intervention was given for 3 weeks and th[4] blood glucose levels were measured using the GOD-PAP method. The data were analyzed using the Kruskal Wallis test and continued with the Mann-Whitney test. The results sh[27]d a significant difference in the mean GDS levels betwe[2] groups p=0.011 (p<0.05), Mann-Whitney test showed K(-) against P1 (ρ=0.009), K(-)[24]inst P2 (ρ=0.016) , K(-) to P3 (ρ=0.028), and P1 to P2 (ρ=0.028). There was no statistically significant difference in blood glucose levels between the control and treatment groups.

INTRODUCTION

Metabolic syndrome (SM) is not a disease, but rather reflects a combination of several metabolic causes that are directly related to non-communicable disease. WHO (World Health Organization) states that metabolic syndrome is a grouping of obesity, dyslipidemia, hypertension and diabetes mellitus.[1] The prevalence of dyslipidemia in Indonesia in 2010 was 39.8% with the criteria for total cholesterol levels > 200 mg/dl. Dyslipidemia also often accompanies hyperglycemia and insulin resistance.[2] [15]Diabetes Mellitus (DM), which is also one of the [15]complications of dyslipidemia, is still a global health problem. WHO estimates that 171 million people in the world suffer from diabetes in 2000 and will increase to 366 million in 2030. *The*

International Diabetes Federation (IDF) estimates that the prevalence of DM in Indonesia will increase from 5.1% in 2000 to 6.3% in 2030.[3]

One of the risk factors for dyslipidemia itself is the intake of a high-calorie diet, especially high in fat. The excess calories will be stored by the body as fat reserves. The level of fat in the blood will increase which is marked by an increase in total cholesterol levels, an increase in triglyceride levels, an increase in LDL levels and a decrease in HDL levels. This condition will also trigger the occurrence of diabetes mellitus which is characterized by an increase in blood sugar levels. Rats fed a high-fat diet showed significant results in the pathophysiological analysis of insulin resistance syndrome. Mice fed a diet high in lard showed the most significant obesity and insulin resistance.[4]

The ability of insulin to stimulate glucose metabolism was greatly reduced in the adipose tissue of mice fed a high-fat diet compared to mice fed a low-fat diet. This mechanism could be explained by measuring insulin-binding ability, which effects on 2-deoxyglucose uptake and the main pathway of glucose metabolism and enzyme activity related to lipogenesis in rat adipocytes fed a high-fat diet for 7 days. These results suggest that the reduced glucose metabolic response to insulin in high-fat-fed rat adipocytes is the result of decreased intracellular capacity to use glucose for lipogenesis.[5]

Several prevention and control measures that can be taken related to dyslipidemia and subsequent complications including diabetes mellitus are by adjusting diet, physical activity, weight loss, stopping smoking and adding dietary supplements. Supplements that can currently be used as additions in controlling dyslipidemia are supplements containing phytosterols, soy protein, fiber and *polyunsaturated fatty acids* (PUFA) omega-3. Considering that this dyslipidemia is still uncontrolled, scientific studies related to supplements for the control of dyslipidemia are still developing. One of them is the use of natural ingredients that often become waste, namely fruit peels. [2]

Previous research explained that the content of active compounds in fruit peels, which include *non-edible ingredients,* is actually higher than other *edible ingredients* . It is also found in the skin of the kaffir lime (*Citrus hystrix*). Previous studies both *in vitro* and *in vivo* stated that the content of kaffir lime peel has promising potential as an antioxidant, antibacterial, lowering lipid levels, blood sugar levels, and even as an anticancer agent. The active ingredients of kaffir lime peel include tannins, steroids, triterpenoids, essential oils containing citrate, saponins, polyphenols, citronella essential oil, citronellol, linalool, geraniol, hydroxy citronellal, linalyl acetate, flavonoids, naringin, hesperidin and pectin. [6]

The results of previous studies indicate that citrus flavonoids can be developed as an effective agent for blood glucose regulation. Preceding observe suggests that the flavonoids from citrus peels are powerful α-amylase and α-glucosidase inhibitors. The ability inhibition of C. hystrix

peels against α-amylase at 100 µg/ml concentration became 60.97 ± 3,78 %. α-Amylase inhibitors play a critical role in blockading the discharge of glucose from nutritional source of carbohydrates and lengthening glucose absorption leading to reduced postprandial plasma glucose degrees and similarly decrease postprandial hyperglycaemia. The ability inhibition of C. hystrix pells against α-Glucosidase at 100 µg/ml attention turned into 14.15 ± 2.02%. It was indicates that the flavonoids from citrus peels are powerful α-glucosidase inhibitors. α-Amylase and α-glucosidase inhibitory ability of citrus peels would postpone the degradation of starch and oligosaccharides, which might in flip motive a lower inside the absorption of glucose and therefore inhibit the growth in postprandial blood glucose.[7]

Phenol compounds are claimed to protect the liver, reduce glucose production, retard glucose transport through the intestine and liver, inhibit intestinal glucose absorption, stimulate beta cells to increase insulin secretion and exhibit insulin-like effects. The presence of polyphenolic compounds such as gallic acid, hesperidin, and naringin in citrus fruits indicates a role as anti-diabetic activity. The active compounds associated with lipid levels are tannins, saponins and flavonoids, but further research on this has not been so in-depth. [7,8]

This prompted researchers to study further on the effect of kaffir lime peel extract (*Citrus hystrix*) on blood sugar levels in male wistar rats fed a high-fat diet.

METHODS

This research has received ethical approval by the Health Research Ethics Commission (KEPK) Faculty of Medicine, University of Muhammadiyah Semarang with number 113/EC/FK/2019. The research design used was a *post-test only controlled group design*.

Research subject

25 male wistar rats were obtained and maintained at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences (FMIPA) of the State University of Semarang (UNNES). Mice were adapted for 1 week before being randomized according to inclusion and exclusion criteria. Mice were placed in cages according to the distribution of the treatment groups. The room temperature and conditions were in accordance with the rat maintenance *guidelines* . Rats were given food and drink according to the standard. Mice are avoided from conditions that cause stress and external exposure that will affect the quality of life of the experimental animals.

Making Kaffir Citrus Peel Extract

The extraction process was carried out at the STIFAR Laboratory in Semarang. Kaffir lime peel extract was obtained by maceration method. The kaffir lime peel is separated from the flesh and then dried. The dried kaffir lime peel is then blended. The dry preparation was then dissolved in 90% ethanol for 2 days. The immersion is then processed in the evaporator for 14 hours to obtain

a thick extract. The extract was then analyzed to determine the phytochemical content qualitatively.

Intervention Process on Experimental Animals

Rats were divided into 5 groups *by simple random sampling* with a total of 5 individuals in each group. The negative control group (K-) was not given a diet high in fat and extract, the positive control group (K+) was given additional pork oil at a dose of 3 mg/200grBW rats only, treatment group 1 (P1) was given additional pork oil at a dose of 3 mg/day. 200grBW rats and kaffir lime peel extract at a dose of 35 mg/kgBW rats/day, treatment group 2 (P2) was given additional pork oil at a dose of 3 mg/200grBW rats and kaffir lime peel extract at a dose of 70 mg/kgBW rats/day and the treatment group 3 (P3) was given additional pork oil at a dose of 3 mg/200grBW rats and kaffir lime peel extract at a dose of 140 mg/kgBW rats/day. The intervention was carried out for 3 weeks.

Termination and Blood Sampling

After 3 weeks of treatment, the rats were fasted first and then anesthetized using ether inhalation and blood taken through the orbital sinus. The blood in the microtube was allowed to stand for 30 minutes and then centrifuged for 10 minutes to obtain blood serum. The blood serum of each rat was then taken to the Semarang Health Laboratory Center for an examination of fasting blood sugar (GDP) levels using a box filled with ice so that the temperature was maintained around 20-40 C.

Blood Glucose Level Check

Blood glucose levels were checked at the Semarang Health Laboratory using the GOD-PAP (Glucose Oxsidase – Peroxidase Aminoantypirin) method.

Data analysis

Blood glucose level data were tested for normality and homogeneity using Saphiro Wilk, because the data was not normal and homogeneous, a test using Kruskal Wallis was used to determine the difference in mean blood glucose levels between groups, and continued with the Mann-Whitney test to determine which groups were different. Meaning.

RESULTS

The study was conducted in February-March 2020. The basic phytochemical test analysis of kaffir lime peel extract is shown in table 1, showing that orange peel extract contains alkaloids, flavonoids, phenolic compounds, tannins, saponins and terpenoids/steroids.

Table 1. Results of Phytochemical Analysis of Kaffir Citrus Peel Extract.

No	Parameter	Results
1	Alkaloids	+
2	Flavonoids	+
3	Phenolic	+
4	Tannins	+

5	Saponins	+
6	Terpenoids/steroids	+

Examination of Blood Glucose Levels was carried out by the GOD-PAP method. The average results of glucose levels for each group are shown in table 2.

Table 2. Descriptive Data on Mean Blood Glucose Levels (mg/dL) in each group (N=25).

Group	Average Blood Glucose Level (mg/dL)
K(-) negative control	45.34
K(+) positive control	76.2
Treatment 1 (P1)	92.58
Treatment 2 (P2)	83.52
Treatment 3 (P3)	78.98

Based on table 2, it can be seen that the highest average blood glucose level of male wistar rats of 92.58 mg/dl was in group P1 (rats with high fat diet and 35 mg/200grBB/day extract). while the lowest average blood glucose level was 45.34 mg/dl in group K (-) (rats fed standard diet without kaffir lime peel extract).

Table 3. Results of Differences in Mean Blood Glucose Levels between the treatment and the control group (N=25).

Group	Kruskal Wallis	Mann-Whitney (p-value)				
		K-	K+	P1	P2	P3
K-	0.011*	-	0.076	0.009*	0.016*	0.028*
K+		0.076	-	0.076	0.754	0.602
P1		0.009*	0.076	-	0.028*	0.117
P2		0.016*	0.754	0.028*	-	0.347
P3		0.028*	0.602	0.117	0.347	-

* P<0.05 (significant)

Based on table 3, the results of the Mann-Whitney test there are significant differences in group K(-) with P1 (ρ=0.009), group K(-) with P2 (ρ=0.016), group K(-) with P3 (ρ=0.028), and P1 with P2 (ρ=0.028), while for group K(+) with K(-) (ρ=0.076), group K(+) with P2 (ρ=0.754), group K(+) with P3 (ρ=0.602) so that there was no significant difference between the K(+) group and the K(-), P2, and P3 groups.

DISCUSSION

This study was aimed at assessing the effect of kaffir lime peel extract on blood glucose levels in wistar rats fed a high-fat diet. The administration of kaffir lime peel extract showed that

there was a significant difference in blood glucose levels of wistar rats between the negative control group and the treatment group, but the rat model fed a high-fat diet had no significant difference with the negative control group indicating that the dose of pork oil intended to add calorie intake is not optimal in increasing blood sugar levels in rats. Although blood glucose levels tended to decrease with increasing dose, blood sugar levels in the treatment group were still higher than the positive control group. This can be influenced by various factors, including the influence of other compounds contained in the lime peel extract itself.

Metabolic disorder, which includes hypertension, diabetes mellitus, dyslipidemia, and obesity, represents a major global health concern due to increased morbidity and mortality.[9] Oxidative stress and inflammation are involved in the pathogenesis of the disorder.[10] The rind extract of kaffir lime, which is rich in flavonoids, possesses high antioxidant. It also demonstrates lipase-inhibiting activity which is beneficial for the treatment of obesity, angiotensin-converting enzyme-inhibiting property for the management of hypertension, moderate inhibiting activity against α-amylase and α-glucosidase which could be useful in diabetes.[11]

The basic phytochemical test analysis in this study is in line with research conducted by Karlina, 2020, which showed that the ethanolic extract of kaffir lime leaves contains alkaloids, flavonoids, saponins, tannins, and steroids/triterpenoids.[12] The results of previous studies conducted by Sanmuga in 2018 indicate that citrus flavonoids can be developed as an effective agent for blood glucose regulation. Flavonoids from citrus peels are powerful α-glucosidase inhibitors. α-Amylase and α-glucosidase inhibitory capability of citrus peels might put off the degradation of starch and oligosaccharides, which might in turn purpose a lower within the absorption of glucose and consequently inhibit the boom in postprandial blood glucose.[7] Phenol compounds are claimed to defend the liver, lessen glucose manufacturing, retard glucose shipping via the intestine and liver, inhibit intestinal glucose absorption, stimulate beta cells to growth insulin secretion and exhibit insulin-like results. The presence of polyphenolic compounds consisting of gallic acid, hesperidin, and naringin in citrus end result indicates a function as anti-diabetic activity.[8]

The results of phytochemical tests in this study were almost the same as the content of soursop bark, where orange peel extract contained alkaloids, flavonoids, phenolics, tannins, saponins and terpenoids/steroids.[13] Research conducted by Juwita in 2015 indicate that the administration of ethanol extract of soursop bark had a significant effect on reducing cholesterol levels, but had no significant effect on reducing blood glucose levels.[14] It is possible that these compounds can have opposite effects so that the effect of the extract on glucose levels is not statistically significant.

There are many other compounds in kaffir lime peel that still need to be studied further as mentioned above. Although the results of this study still do not strongly support the previous

theories, they have different potential results if the high-fat mouse model is improved. Another limitation of this study is that it has not been able to separate each active compound from kaffir lime peel which in vitro has shown an effect. This can affect the results if there are compounds whose activities are opposite to each other. In addition, other studies are also needed by analyzing various lipid profiles to better understand the role of this kaffir lime peel extract

CONCLUSION

Giving kaffir lime peel extract (*Citrus hystrix*) had no effect on blood glucose levels of wistar rats fed a high-fat diet at a dose of 35 mg/kg BW rats/day, 70 mg/kgBW rats/day and 140 mg/kgBW rats/day.

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