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Raphanus Sativus Leaves Ethanol Extract's Effect on Heart Muscle's Nuclear Factor Kappa B (NFkB) in Diabetic Rats.

Asri Hendrawati[1], Nur Aini Djunet[2]

[1,2)] Department of Biochemistry and Nutrition, Faculty of Medicine, Universitas Islam Indonesia, Yogyakarta, Indonesia

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Correspondence:
097110416@uii.ac.id

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Abstract

Background: Type 2 diabetes mellitus is characterized by hyperglycemia. Hyperglycemia increases free radicals and oxidative stress that damage heart muscle cells. Nuclear factor kappa B (NFkB) is essential in inflammation and cell damage. Radish leaves (Raphanus sativus) are known to have compounds that can control NFkB expression.

Objective: To measure the effect of radish leaves on the expression of NFkB in heart muscle.

Methods: The study was designed in an experimental laboratory posttest control group. The subjects were diabetic male Wistar rats weighing 150-300 grams and were 3-4 months old. Rats were divided into four groups and given treatment orally for 28 days. The 1st group received a placebo. The 2nd group received glibenclamide 5 mg/kg BW/day. The 3rd and 4th groups received 50% and 100% ethanol extract of radish leaves. Heart NFkB was measured using immunohistochemistry.

Results: Radish leaves ethanol extract 50% and 100% reduced NFkB in the heart muscle better than placebo and glibenclamide 5 mg/kg BW ((p=0.000). There was no difference between 50% and 100% radish leaf extract in reducing heart NFkB expression (p=0.876).

Conclusion: Radish leaf extract 50% and 100% reduce NFkB expression better than placebo and glibenclamide 5 mg/kg BW.

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by increased blood sugar levels or hyperglycemia. Globally, there were 425 million people with DM in 2017. The estimated number of DM will increase to 629 million people in 2045. Indonesia ranks sixth in the world for the highest prevalence of diabetes in the world in 2017, with an estimated 10.3 million people suffering from diabetes.[1]

Type 2 DM (T2DM) is the most common type of DM, with about 90% of DM incidents. Insulin resistance is the initial cause of T2DM**,** which increases blood glucose levels. The pancreatic cells (insulin hormone-producing cells in the pancreas) will compensate for this condition. Over time, pancreatic cells cannot long for this because chronic hyperglycemic conditions tend to increase the formation of free radicals (ROS) through various pathways of glucose metabolism. ROS will increase oxidative stress, disrupting pancreatic cell function.[2] Histopathologically, the islets of Langerhans in T2DM patients will change to smaller. Also, qualitative changes such as necrosis, degeneration, and amyloidosis can be observed.[3]

Treatment of T2DM with hypoglycemic agents is less effective in reducing oxidative stress and can cause side effects.[4] Therefore, the popularity of antidiabetic herbs with low side effects has increased recently.[5] The wealth of natural resources in Indonesia, including herbal plants that can be used as a source of treatment**;** one of these plants is radish (Raphanus sativus). According to the Central Statistics Agency, the production of radish plants in Indonesia reaches 22,417 tons per year. Radish is often consumed in various countries, in processed vegetables, or as a spice in food. According to Banihani, the radish plant has antidiabetic activity.[6] Radish can improve insulin sensitivity. The pancreatic histology of DM rats given 10% radish extract for six weeks appears normal compared to the negative control group.[7] The leaves can reduce glucose absorption in the intestines by inhibiting the glucosidase enzyme. The roots reduce fasting blood sugar and oxidative stress through their antioxidant activity. The antioxidant activity in radish roots comes from the content of flavonoid compounds. These compounds are known to be able to scavenge free radicals (radical scavenging) and reduce ROS formation (by binding to iron.[8]

Hyperglycemia will increase the expression and activity of NFkB. The transcription factor NFkB will translocate from the cytoplasm to the nucleus and increase the production of inflammatory mediator proteins such as interleukin 6. Interleukin-6 is a factor that disrupts glucose homeostasis in diabetes mellitus and promotes pancreatic damage and insulin resistance. The NFkB plays an essential role in the pathogenesis of T2DM, so if its activity is reduced, the progression of DM and the onset of cell damage that causes various complications can be prevented.[9]

Research on the antidiabetic effect of radish leaves is limited, but several studies have shown that the flavonoid compounds of radish leaves exceed the roots. According to Goyeneche et al., the total flavonoid content in radish leaves is 4x higher than in radish roots. The flavonoid compounds in radish leaves include quercetin, kaempferol, and pelargonidin. The high flavonoid compound content makes the radish leaves' antioxidant capacity 3.6 times

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higher than the roots. The administration of ethanol extract from radish leaves containing this antioxidant can prevent cell damage due to oxidative stress conditions in T2DM, including reducing the expression of NFkB.[10]

METHODS

This study was an experimental study with **A** posttest**-**only control group design. The ethical clearance is certified by The Health Research Ethics Committee, Faculty of Medicine Universitas Islam Indonesia**,** by number 8/Ka.Kom.Et/70/KE/I/2020.

The animal subjects were male Wistar rats. The inclusion criteria were male Wistar (Rattus norvegicus) strain rats weighing 150-300 grams, 3-4 months old, healthy, without physical disabilities, and never used for research. The sample size was determined using the Resource Equation formula conducted by Charan and Kantharia (2013)[11], E= N-T. Our study used four treatment groups, so the sample calculation was:

10-20 = N – T

10-20 = N – 4

N = 14-24

N= Total number of animals

T= Total number of groups

Based on this formula, the optimal number for our study was 14-24 rats. All rats were induced using a nicotinamide dose of 120 mg/ kgBW and streptozotocin (STZ) 60 mg/kg BW dissolved in 100 mM cold citrate buffer at pH 4.5 intraperitoneally. Blood glucose levels were measured one week after induction, and rats with blood sugar levels ≥200 mg/dL were included in the study. We divided them into four groups, each with four rats. The first group was a diabetic rats given placebo (plain water), the second group was a diabetic rats given glibenclamide 5 mg/kgBW, the third group was a diabetic rats given 50% radish extract, and the fourth group was a diabetic rats given 100% radish extract. All treatments were given orally for 28 days. After the treatment, the heart was taken and made into a paraffin block. Then, a slide preparation was made, and immunohistochemistry staining was performed to measure the percentage of NFkB. Data on the percentage of heart muscle NFkB was obtained by counting the number of cells expressing NFkB in their nucleus divided by the total number of cells in 1 field of view and then multiplied by 100%. The calculation of the percentage of NFkB for each sample was carried out in 5 fields of view. Then, the average percentage value is taken as the final data for the percentage of NFkB. Cells expressing NFkB in their nuclei are shown in brown in their nuclei. Meanwhile, cells that do not express NFkB in their nuclei are shown in blue in their nuclei.

RESULTS

We used the heart tissue paraffin blocks for IHC examination. The distribution of groups is shown in Table 1. The rats' fasting blood

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sugar was eligible and could be given treatment until completion.

After the end of treatment, rats were decapitated, and the hearts and tissue of rats were used for immunohistochemical (IHC) staining. We measured the proportion of NFkB in the heart muscle. The proportion was calculated by counting the number of brown cells that express NFkB in a visual field. The examination was held in 5 fields, and the average was calculated. The proportion of NFkB was normally distributed and had the same variance, so the differences between groups were tested using One-way ANOVA. The data on the average percentage of heart muscle NFkB are shown in Table 2. The results showed that the two groups had significantly different NFkB proportions (p=0.000). To find out which groups have significantly different NFkB percentages, a post hoc test was performed using the LSD test (Table 3).

Table 1. Distribution of treatment groups

Group name	Group description
K1	Diabetic rats were given a placebo (plain water) for 28 days.
K2	Diabetic rats were given glibenclamide (synthetic hypoglycemic agent) tablets, which were crushed and dissolved in DMSO at a dose of 5 mg/kg BW once per day orally for 28 days.
K3	Diabetic rats were given radish leaf extract with a concentration of 50% per day orally for 28 days.
K4	Diabetic rats were given radish leaf extract with a concentration of 100% per day orally for 28 days.

Table 2. The average proportion of heart muscle NFkB (%)

Group name	Heart muscle NFkB proportion (%)	P*
K1	17.74±2.44	0.000
K2	14.87±2.25	
K3	9.71±1.63	
K4	8.55±2.43	

**One Way ANOVA test is meaningful if the p-value <0.05.*

Table 3. The results of the LSD post hoc test

	Groups	P*
K1	K2	0.023
	K3	0.041
	K4	0.027
K2	K3	0.032
	K4	0.015
K3	K4	0.876

**LSD test is meaningful if the p-value <0.05.*

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There was a significant difference in the proportion of NFkB in the heart muscle cell nucleus between K1 with, K2, K3, and K4. There was a significant difference in the proportion of NFkB in the heart muscle cell nucleus between K2 with K1, K3, and K4. There was a significant difference in the proportion of NFkB in the heart muscle cell nucleus between K3 with K1 and K2. There was a significant difference in the proportion of NFkB in the heart muscle cell nucleus between K4, K1, and K2. There was no significant difference in the proportion of NFkB in the heart muscle cell nucleus between K3 and K4.

DISCUSSION

This study showed an increase in the expression level of heart muscle NFkB in DM rats compared to healthy rats. According to previous studies, NFkB is under-expressed in healthy cell nuclei.[12] In an earlier study, researchers examined the level of NFkB expression in the heart muscle cells of healthy mice and found that the mean percentage of NFkB was 3.69±0.29. In DM conditions, an increase in oxidative stress damages cells, thereby increasing the inflammatory process by activating NFkB. A previous study stated that the expression of NFkB increased in hyperglycemic conditions for one month**,** and it was tested on experimental animals.[13,14]

This study found that the DM rat group given glibenclamide at a dose of 5 mg/kg BW/day for four weeks had a significantly lower average level of NFkB expression in the heart muscle cell nucleus than that given a placebo. Previous studies stated that sulfonylurea drugs such as glimepiride and glipizide can increase the transcriptional activity of PPAR γ because it acts as a ligand. Glibenclamide belongs to the sulfonylurea class of oral antihyperglycemic drugs.[15] PPARγ will bind to NFkB, preventing translocation of NFkB to the cell nucleus and preventing its activation.[16,17]

The DM rat group that was given radish leaf extract at a concentration of 50% or 100% per day orally for 28 days had a significantly lower average level of NFkB expression in heart muscle cell nuclei compared to those given placebo or glibenclamide at a dose of 5 mg/kg BW/day. Radish leaves contain several flavonoid antioxidants, including quercetin.[18] Previous research stated that quercetin is an anti-inflammatory agent that inhibits the activity of pro-inflammatory cytokines.[19] Another study also indicated that quercetin inhibits NFkB activation, thus reducing the production of inflammatory cytokines.[20] In this study, there was no significant difference between radish leaf extract concentrations of 50% and 100% for 28 days in reducing the level of NFkB expression in rats' heart muscle cell nucleus.

CONCLUSION

Radish leaf extract with concentrations of 50% and 100% for 28 days reduced the percentage of NFkB in rats' heart muscle cell nucleus significantly better than placebo and glibenclamide 5 mg/kg BW. Radish leaves effectively prevent inflammation by reducing NFkB activity in the heart muscle and preventing damage to heart muscle cells.

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