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RESEARCH ARTICLE
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Raphanus Sativus Leaves Ethanol Extract's Effect on Heart Muscle's Nuclear Factor Kappa B in Diabetic Rats

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Abstract:

Type 2 diabetes mellitus is characterized by hyperglycemia. Hyperglycemia increases oxidative stress that damage cells including in heart muscle. Oral hypoglycemic drugs cant reduce the expression of nuclear factor kappa B (NFkB) which plays an important role in inflammation and cell damage. Radish leaves (Raphanus sativus) are known to have compounds that can reduce the expression of NFkB.This study aimed to determine the effect of radish leaves on the expression of NFkB in heart muscle. The study design was an experimental laboratory post-test control group design. The subjects were DM male Wistar rats (Rattus norvegicus), weight 150-300 grams, 3-4 months old. Rats were divided into four groups and took the treatment orally for 28 days. The 1st group got plain water. The 2nd group got glibenclamide 5 mg/kg BW/day. The 3rd and 4th groups got 50% and 100% ethanol extract of radish leaves. After the end of treatment, the heart's tissue was taken for immunohistochemical (IHC) staining to measure the proportion of NFkB. The intervention of ethanol extract of radish leaves 50% and 100% for 28 days reduced the percentage of NFkB in the heart muscle cell of rats than placebo and glibenclamide 5 mg/kg BW ((p=0.000). There was no difference between radish leaf extract concentration of 50% and 100% for 28 days in reducing the expression level of NFkB in the heart muscle cell of rats (p=0.876).This research has not been done before and is important for the treatment of diabetes mellitus in the future.

Keywords: Raphanus sativus; nuclear factor kappa B; heart muscle; diabetes mellitus, rats

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.[2] Insulin resistance is the initial cause of T2DM 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. The ROS will increase oxidative stress, so the pancreatic cell function is disrupted.[2] Histopathologically the islets of Langerhans in T2DM patients will change to be smaller. And also qualitatively changes such as necrosis, degeneration, and amyloidosis.[3]

Treatment of T2DM with hypoglycemic agents is less effective in reducing oxidative stress and can cause side effects.[4,5] Therefore, the popularity of antidiabetic herbs that have low side effects increase in recent years.[5] The wealth of natural resources in Indonesia, including herbal plants that can use as a source of treatment, one of these plants is radish (Raphanus sativus). According to the Central Statistics Agency (2018), 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 (2017), the radish plant has antidiabetic activity. Radish can improve insulin sensitivity. The pancreatic histology of DM rats given 10% radish extract for 6 weeks appears normal compared to the negative control group.[6] The leaves can reduce glucose absorption in the intestines by inhibiting the glucosidase enzyme. The roots are known to reduce fasting blood sugar and reduce oxidative stress conditions 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).[7]

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 important 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.[8]

Research on the antidiabetic effect of radish leaves is limited, several studies show that the flavonoid compounds of radish leaves exceed the roots. According to Goyeneche et al., (2015), the total flavonoid content in radish leaves is 4x higher than in radish roots. The flavonoid compounds contained in radish leaves include quercetin, kaempferol, and pelargonidin. The high content of flavonoid compounds makes the antioxidant capacity of radish leaves 3.6 times higher than the roots. The administration of ethanol extract of radish leaves containing this antioxidant can prevent cell damage due to oxidative stress conditions in T2DM, including reducing the expression of NFkB.[9]

Research Method

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 had never been used for research. The determination of the sample size was by the Resource Equation formula conducted by Charan and Kantharia (2013) (10), E= N-T.

Based on this formula, the optimal number for our study was 14-24 rats. We divided them into four groups and each group consisted of four rats. The first group was a negative control given plain water, the second group was positive control given glibenclamide 5 mg/kg BW, the third group received 50% radish extract, and the 4th group received 100% radish extract. All treatments were given orally for 28 days. After the treatment finished, the rats' heart was taken for immunohistochemistry (IHC) examination to measure the percentage of NFkB IHC technique done by paraffin block preparation, antigen retrieval, selection and preparation of antibody and reagents, incubation, washing, and counterstaining The proportion was calculated by counting the number of brown cells that express NFkB in a visual field. The examination was held in 5 different fields, and the average was calculated.

The distribution of groups is shown in Table 1. The rats fasting blood sugar was eligible, then they could be given treatment until completion.

Table 1. Distribution of treatment groups

Group name	Group description
K1	The negative control group, DM rats given plain water for 28 days
K2	The positive control group, DM 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	The experimental group were given radish leaf extract with a concentration of 50% per day orally for 28 days
K4	The experimental group were given radish leaf extract with a concentration of 100% per day orally for 28 days

Results

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 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.

The K1 group got plain water. The K2 group got glibenclamide 5 mg/kg BW/day. The K3 got 50% ethanol extract from radish leaves, and The K4 group had 100% ethanol extract from radish leaves.

There was a significant difference in the proportion of NFkB in the heart muscle cell nucleus between K3 and K1 and K2. There was a significant difference in the proportion of NFkB in the heart muscle cell nucleus between K4 and K1 and K2. There was no significant difference in the proportion of NFkB in the heart muscle cell nucleus between K3 and K4 (p=0.876).

Discussion

In this study, the results showed that there was an increase in the expression level of heart muscle NFkB in DM rats when compared to healthy rats. According to previous studies, NFkB is under-expressed in healthy cell nuclei.[11] In a previous study, NFkB expression in the heart muscle cells of healthy rats was 3.69±0.29. In DM conditions, there is an increase in oxidative stress that damages cells, thereby increasing the inflammatory process through the activation of NFkB. In a previous study, it was stated that the expression of NFkB increased in hyperglycemic conditions for 1 month which was tested on experimental animals.[12]

In this study, it was found that the DM rat group that was given glibenclamide at a dose of 5 mg/kg BW/day for 4 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.[13] PPAR γ will bind to NFkB, preventing translocation of NFkB to the cell nucleus and preventing its activation.[14]

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. Previous research stated that quercetin is an anti-inflammatory agent by inhibits the activity of pro-inflammatory cytokines.[15] Another study also stated that quercetin inhibits NFkB activation reducing the production of inflammatory cytokines.[16] 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. It means that both doses could effectively reduce the NFkB level.

Conclusions

Radish leaves extract with concentrations of 50% and 100% for 28 days reduced the percentage of nuclear factor kappa B in the heart muscle cell nucleus of rats significantly better than placebo and glibenclamide 5 mg/kg BW.

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