

## Quercetin reduce cardiomyocytes damage in type 2 diabetic rats

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### ABSTRACT

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#### BACKGROUND

Diabetes mellitus (DM) is a group of metabolic diseases which are characterized by hyperglycemia, resulting in various complications. A major macrovascular complication of DM is cardiac failure due to cardiomyopathy. Hyperglycemia increases oxidative stress, so an oxidative-stress reducing therapeutic agent is required, e.g. the antioxidant quercetin. The aim of this study was to assess the effects of quercetin in reducing damage to cardiomyocytes of type 2 diabetic rats.

#### METHODS

This research is an experimental study using 40 rats. With simple random allocation, rats were divided into eight groups, then type 2 diabetes mellitus was induced using streptozotocin (5 rats per group). The test material was quercetin given at doses of 5, 20 and 80 mg/kgBW/day orally for 4 weeks. Each single dose of quercetin was given in combination with glibenclamide 5 mg/kgBW/day. After 4 weeks the rats were decapitated and the cardiac tissues taken to quantify the percentage of cell damage after hematoxylin-eosin staining (HE).

#### RESULTS

Quercetin at a dose of 80 mg/kgBW/day can lower cardiomyocyte damage better than quercetin at doses of 5 or 20 mg/kgBW/day. A combination of quercetin and glibenclamide can significantly lower levels of cardiomyocyte damage better than quercetin without glibenclamide ( $p < 0.05$ ).

#### CONCLUSION

Quercetin at a dose of 80 mg/kgBW/day with or without glibenclamide can lower damage to cardiomyocytes of type 2 diabetic rats. Thus quercetin might serve as a valuable protective agent in cardiovascular inflammatory diseases in diabetic rats.

**Keywords:** Quercetin, glibenclamide, cardiomyocytes, type 2 diabetes mellitus, rats

## ***Kuersetin menurunkan kerusakan sel otot jantung pada tikus diabetes melitus tipe 2***

### **ABSTRAK**

#### **LATAR BELAKANG**

Diabetes melitus (DM) merupakan kelompok penyakit metabolik yang ditandai hiperglikemia. Berbagai komplikasi karena DM disebabkan oleh hiperglikemia. Komplikasi makrovaskuler utama DM adalah gagal jantung karena kardiomiopati. Hiperglikemia menyebabkan peningkatan stress oksidatif sehingga diperlukan terapi untuk menurunkan stress oksidatif seperti antioksidan kuersetin. Penelitian ini bertujuan untuk menilai efek kuersetin dalam menurunkan kerusakan sel otot jantung tikus DM tipe 2.

#### **METODE**

Penelitian eksperimental menggunakan kontrol dilakukan pada 40 ekor tikus. Secara alokasi random sederhana, tikus dibagi menjadi 8 kelompok yang diinduksi menggunakan streptozotocin menjadi DM tipe 2 (5 ekor per tikus kelompok). Bahan uji adalah kuersetin dosis 5, 20 dan 80 mg/kgBB/hari peroral selama 4 minggu. Masing-masing dosis kuersetin diberikan tunggal dan kombinasi dengan glibenklamid 5 mg/kgBB/hari. Perlakuan diberikan selama 4 minggu. Kemudian tikus didekapitasi dan diambil jantungnya untuk dihitung persentase kerusakan selnya setelah pewarnaan hematoksilin-eosin (HE).

#### **HASIL**

Kuersetin dosis 80 mg/kgBB/hari menurunkan kerusakan sel otot jantung lebih baik dari kuersetin dosis 5 atau 20 mg/kgBB/hari. Pemberian kombinasi kuersetin bersama glibenklamid menurunkan tingkat kerusakan sel otot jantung lebih baik secara bermakna dibandingkan kelompok tanpa kombinasi dengan glibenklamid ( $p < 0,05$ ).

#### **KESIMPULAN**

Kuersetin dosis 80 mg/kgBB/hari dengan atau tanpa kombinasi glibenklamid mampu menurunkan kerusakan sel otot jantung tikus DM tipe 2. Penelitian ini membuktikan kuersetin merupakan agen yang bermanfaat untuk mencegah kerusakan sel otot jantung pada tikus DM tipe 2.

**Kata kunci:** Kuersetin, glibenklamid, sel otot jantung, diabetes melitus tipe 2, tikus

## **INTRODUCTION**

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia, causing damage, dysfunction, and failure of organs, such as the eyes, kidneys, heart, and blood vessels.<sup>(1)</sup> In 2005 DM had reportedly affected 135 million world inhabitants (around 3% of the world population), including the urban and rural poor.<sup>(2)</sup> The number of DM patients in the world is projected to reach 522 million in the year 2030.<sup>(3)</sup> In Indonesia there are around 8.4 million

people with DM or around 5% of its population and this number is estimated to reach around 21.3 million in 2030.<sup>(4)</sup>

Chronic hyperglycemia in DM causes glucose autooxidation into free radicals, such as superoxides that can easily react with cells, so causing cell damage and even cell death.<sup>(5)</sup> Diabetes mellitus is a high risk factor for heart failure, similar to coronary heart disease and hypertension. Heart failure in DM is mainly caused by death of cardiomyocytes (diabetic cardiomyopathy). The major cause of cardiac

muscle death is principally attributed to increased production of reactive oxygen species (ROS) in DM patients.<sup>(6)</sup>

Currently, administration of oral hypoglycemic agents is less effective in reducing oxidative stress in DM patients.<sup>(5)</sup> Therefore, administration of antioxidants such as quercetin plays an important role in the treatment of DM to protect the organs, including cardiac muscle, against free radical-induced damage.

Quercetin, a flavonoid compound that has been intensively studied in vivo and in vitro, exerts a hypoglycemic effect and reduces the risk of obesity.<sup>(7)</sup> Quercetin and other flavanoids have been shown to increase the synthesis and activity of antioxidant enzymes, such as catalase, in reducing oxidative stress.<sup>(7)</sup> Quercetin has a protective effect on pancreatic  $\beta$ -cells and reduces oxidative stress. Quercetin increases insulin secretion and protects pancreatic  $\beta$ -cells against oxidative damage via the extracellular signal-related kinase (ERK 1/2) pathway.<sup>(8)</sup>

The aim of the present study was to evaluate the ability of quercetin to prevent cardiomyocyte damage in type 2 diabetic rats.

## METHODS

### Design of the study

This was an experimental study of post test control group design. The study period was between February and June 2014.

### Experimental animals

The experimental animals used were 40 three-month old male Wistar rats weighing between 150 and 250 grams each. The sample size was calculated with Federer's formula:  $(n-1)(t-1) \geq 15$ , giving for 8 groups the minimal number of 4 animals per group.<sup>(9)</sup> This study used 5 animals per group. The animals were kept and tested in the Central Inter-University Laboratory of Gadjah Mada University.

### Experimental protocol

The rats were assigned by simple random allocation into 8 groups of 5 rats, in whom type

2 DM was induced by a single intraperitoneal injection of streptozotocin at a dose of 60 mg/kgBW, dissolved in 100 mM cold citrate buffer at pH 4.5. Fifteen minutes after streptozotocin administration, the rats were given a single intraperitoneal injection of nicotinamide at a dose of 120 mg/kgB. One week after streptozotocin induction, the fasting blood glucose concentration of the rats was determined. Rats with a fasting blood glucose of more than 126 mg/dL were considered to be diabetic.<sup>(10)</sup> The diabetic rats were then subjected to the interventions, where group 1 (K1) received daily oral sodium carboxymethyl cellulose (CMC) as placebo, group 2 (K2) diabetic rats received glibenclamide 5 mg/kgBW/day once daily,<sup>(11)</sup> group 3 (K3) were given quercetin 5 mg/kgBW/day, group 4 (K4) diabetic rats quercetin 20 mg/kgBW/day,<sup>(12)</sup> group 5 (K5) diabetic rats quercetin 80 mg/kgBW/day, group 6 (K6) quercetin 5 mg/kgBW/day plus glibenclamide 5 mg/kgBW/day, group 7 (K7) diabetic rats quercetin 20 mg/kgBW/day plus glibenclamide 5 mg/kgBW/day, and group 8 (K8) received quercetin 80 mg/kgBW/day plus glibenclamide 5 mg/kgBW/day. The interventions were administered daily to the diabetic rats by a single gavage. The interventions were administered for 4 weeks.<sup>(12)</sup>

### Histopathologic examination

After 4 weeks of treatment, all rats were decapitated and their hearts taken for preparation of hematoxylin-eosin (HE) stained sections. Normal cardiomyocytes showed centrally located oval nuclei, surrounded by regular miofibrils. Damaged cells were characterized by alterations in the size and shape of the nuclei, which became more rounded or square, surrounded by fibrotic tissue.<sup>(13)</sup> Cardiac muscle damage was expressed as the percentage (%) of square/rounded nuclei in five high power (400x) fields under the light microscope.<sup>(14)</sup>

### Determination of fasting blood glucose

The rats were fasted for 12 hours before their blood was collected for determination of

Table 1. Means of body weights and fasting blood glucose concentrations by intervention group at baseline

	K1 (n=5)	K2 (n=5)	K3 (n=5)	K4 (n=5)	K5 (n=5)	K6 (n=5)	K7 (n=5)	K8 (n=5)	P
Weight (grams)	239.33 ± 21.56	219.17 ± 15.75	218.50 ± 11.98	218.67 ± 24.71	223.67 ± 14.24	223.67 ± 20.12	223.67 ± 18.18	207.00 ± 18.17	0.528
Fasting blood glucose (mg/dL)	170.90 ± 45.78	172.76 ± 23.94	141.22 ± 28.74	169.00 ± 24.93	228.63 ± 43.77	182.54 ± 31.95	195.88 ± 3.03	200.79 ± 25.58	0.005*

Notes: \*significant one-way ANOVA if  $p < 0.05$ . K1: diabetic rats on placebo/day, K2: diabetic rats on glibenclamide 5mg/kgBW/day, K3: diabetic rats on quercetin 5 mg/kgBW/day, K4: diabetic rats on quercetin 20 mg/kgBW/day, K5: diabetic rats on quercetin 80 mg/kgBW/day, K6: diabetic rats on quercetin 5 mg/kgBW/day and glibenclamide 5mg/kgBW/day, K7: diabetic rats on quercetin 20 mg/kgBW/day and glibenclamide 5mg/kgBW/day, K8: diabetic rats on quercetin 80 mg/kgBW/day and glibenclamide 5mg/kgBW/day.

blood glucose. The blood was drawn from the orbital sinus. The blood glucose concentration of the rats was measured using a spectrophotometer. This instrument is based on the principle of oxidation of glucose by glucose oxidase, resulting in release of hydrogen peroxide, which then reacts with 4-aminoantipyrine and phenol. This reaction is catalyzed by peroxidases and produces the brownish-yellow quinoneimin. Color intensity is measured at 500 nm wavelength. The intensity of the color produced is proportional to the glucose concentration.

#### Data analysis

The between-group differences in cardiac cell damage was tested by one-way ANOVA, followed by post hoc LSD.

#### Ethical clearance

This study received ethical clearance from the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Gadjah Mada University, Yogyakarta, with reference number KE/FK/90/EC.

#### RESULTS

Although there were no significant differences in the weight of the rats ( $p=0.528$ ) between the intervention groups at baseline, there were significant differences in fasting glucose

concentrations ( $p=0.005$ ) (Table 1). The results of multiple comparison LSD showed differences in fasting blood glucose concentrations between K1 and K5, between K1 and K7, and between K6 and K8 (data not shown).

The degree of cardiomyocyte damage was determined by microscopic study of cardiomyocyte histology after hematoxylin-eosin (HE) staining. Normal cardiomyocytes were recognized by their centrally located oval nuclei, with regular miofibrils surrounding them. Damaged cells were characterized by alterations in the size and shape of the cell nuclei, which became more rounded or square, surrounded by an accumulation of fibrotic tissue. The histologic picture of HE-stained normal and damaged cardiomyocytes are shown in Figures 1 and 2, respectively.

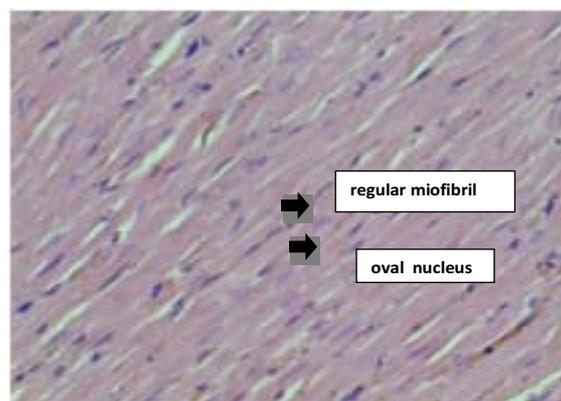


Figure 1. Healthy rat cardiomyocytes stained with HE, showing oval cell nuclei surrounded by regular miofibrils

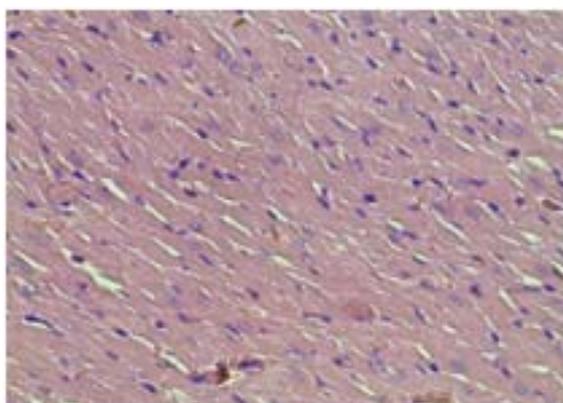


Figure 2. Damaged cardiomyocytes of diabetic rats receiving placebo (HE stain), showing many cell nuclei with more rounded shapes and fibrosis of miofibrils around the nuclei without normal miofibril morphology

After 4 weeks of intervention, there were no significant between-group differences in mean body weight of the rats ( $p=0.116$ ). However, there were significant differences in fasting blood glucose concentrations between intervention groups after 4 weeks of intervention ( $p=0.003$ ) (Table 2). Multiple comparison LDS analysis showed significant differences in fasting blood glucose concentration between K1 and K4 and between K1 and K8. There were also significant differences in fasting blood glucose concentrations between K3 versus K4 and K8, as well as between K4 and K6, and between K6 and K8 (data not shown). In the determination

of cardiomyocyte damage, there were significant differences in the mean percentages between the intervention groups ( $p=0.000$ ). The lowest mean percentage of cardiomyocyte damage was found in K8 and the highest in K1 (Table 2).

## DISCUSSION

The present study found a significant weight loss in the group of rats with induced DM, one of the clinical signs of which is unexplained weight loss. In type 2 DM there is insulin resistance resulting in decreased cellular glucose uptake. The cells cannot utilize sufficient amounts of glucose as a source of energy, inducing a compensatory utilization of non-carbohydrates as energy source, such as fat and protein reserves. This leads to a compensatory increase in lipolysis and a compensatory decrease in lipogenesis, thereby resulting in weight loss.<sup>(15)</sup> The present study found that quercetin alone at 80 mg/kgBW/day was effective in reducing the blood glucose concentration of diabetic rats, as compared with 5 mg/kgBW/day or 20 mg/kgBW/day. Quercetin has previously been shown to significantly reduce blood glucose concentration in patients with DM.<sup>(16)</sup> In another study, oral quercetin at a dose of 100 mg/kgBW/day given for 7 weeks significantly reduced fasting and 2-hour postprandial blood glucose concentration

Table 2. Means of weight, fasting blood glucose, and cardiac cell damage after 4 weeks of intervention by intervention group

	K1 (n=5)	K2 (n=5)	K3 (n=5)	K4 (n=5)	K5 (n=5)	K6 (n=5)	K7 (n=5)	K8 (n=5)	P
Weight (grams)	201.67 ± 15.58	203.33 ± 18.07	188.50 ± 16.35	186.33 ± 23.64	196.67 ± 19.89	189.83 ± 29.46	192.83 ± 9.43	170.33 ± 18.07	0.116
Fasting blood glucose (mg/dL)	234.41 ± 55.95	234.51 ± 47.52	216.30 ± 43.96	216.56 ± 19.88	188.72 ± 23.55	200.50 ± 3.16	180.77 ± 13.09	159.66 ± 6.81	0.003*
Cardiac cell damage (%)	23.20 ± 2.73	15.47 ± 0.59	16.87 ± 2.14	11.74 ± 0.54	7.23 ± 1.33	12.93 ± 1.13	9.25 ± 1.09	5.32 ± 0.38	0.000*

Notes: \*significant one-way ANOVA if  $p<0.05$ . K1: diabetic rats on placebo/day, K2: diabetic rats on glibenclamide 5mg/kgBW/day, K3: diabetic rats on quercetin 5 mg/kgBW/day, K4: diabetic rats on quercetin 20 mg/kgBW/day, K5: diabetic rats on quercetin 80 mg/kgBW/day, K6: diabetic rats on quercetin 5 mg/kgBW/day and glibenclamide 5mg/kgBW/day, K7: diabetic rats on quercetin 20 mg/kgBW/day and glibenclamide 5mg/kgBW/day, K8: diabetic rats on quercetin 80 mg/kgBW/day and glibenclamide 5mg/kgBW/day.

in diabetic rats. The ability of quercetin in reducing blood glucose concentration is thought to be due to its ability to inhibit the enzyme  $\alpha$ -glucosidase that plays a role in carbohydrate digestion in the small intestine.<sup>(17)</sup>

The group of diabetic rats given a combination of quercetin 20 mg/kgBW/day and glibenclamide 5 mg/kgBW/day and that given quercetin 80 mg/kgBW/day plus glibenclamide 5 mg/kgBW/day had significant reductions in mean fasting blood glucose concentration. This indicates that the combination of quercetin 20 mg/kgBW/day and glibenclamide is more effective in decreasing blood glucose concentration than glibenclamide 5 mg/kgBW/day or quercetin alone at 20 mg/kgBW/day. A previous study showed that a combination of honey (as antioxidant) and glibenclamide more effectively decreased blood glucose concentration and oxidative stress, as compared with glibenclamide only. The study showed that glibenclamide alone was not significant in preventing pancreatic damage from oxidative stress.<sup>(18)</sup>

In our study, oral quercetin at 80 mg/kgBW/day for 4 weeks and the combination of quercetin 20 mg/kgBW/day or 80 mg/kgBW/day with glibenclamide 5 mg/kgBW/day were effective in decreasing fasting blood glucose concentration of the rats.

In the present study it was found that quercetin 5 mg/kgBW/day was capable of a better reduction of the percentage of cardiomyocyte damage than placebo, being equivalent to glibenclamide 5 mg/kgBW/day. Quercetin at a dose of 20 mg/kgBW/day decreased cardiomyocyte damage better than quercetin 5 mg/kgBW/day, glibenclamide 5 mg/kgBW/day or placebo, but was equivalent to the combination of quercetin 5 mg/kgBW/day plus glibenclamide 5 mg/kgBW/day.

Quercetin may protect cardiomyocytes against damage, because of its ability to decrease oxidative stress in diabetic rats. In a previous study it was found that quercetin at doses of 50

mg/kgBW/day and 80 mg/kgBW/day for 45 days was capable of significantly decreasing oxidative stress in diabetic rats.<sup>(19)</sup> Quercetin is able to promote the activity of the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) to enhance production of endogenous antioxidants capable of decreasing free radicals.<sup>(20)</sup> In the present study, quercetin at a dose of 80 mg/kgBW/day was the best in decreasing cardiomyocyte damage than quercetin at lower doses, with or without glibenclamide.

The administration of quercetin 5 mg/kgBW/day, 20 mg/kgBW/day, or 80 mg/kgBW/day in combination with glibenclamide 5 mg/kgBW/day was shown to significantly decrease cardiomyocyte damage to a higher degree than did the quercetin doses without glibenclamide. This is due to the ability of quercetin in decreasing the numbers of free radicals that damage the cardiomyocytes, assisted by the action of glibenclamide in reducing blood glucose concentration, so decreasing the numbers of free radicals produced by hyperglycemia. A previous study stated that combining standard hypoglycemic drugs with antioxidants was more effective in decreasing free radicals and oxidative stress.<sup>(18)</sup>

From our study results, it is hoped that quercetin may be used as adjunctive treatment for type 2 DM, so decreasing its complications and improving the quality of life of the patients. Before this is possible, however, attention should be given to other variables capable of affecting quercetin bioavailability, e.g. its absorption and distribution, which are different in experimental animals and humans.

A limitation of the present study was the lack of attention to the amount of feed consumed by the experimental animals, which may affect the bioavailability of quercetin as well as the clinical condition of the diabetic animals. During the intervention, the type and amount of feed given to each of the rats was identical, but some rats ate all their daily feed, whereas others left some of their feed untouched.

## CONCLUSIONS

Quercetin at a dose of 80 mg/kgBW/day protected cardiomyocytes better against damage in type 2 diabetic rats than did quercetin at doses of 5 and 20 mg/kgBW/day, glibenclamide 5 mg/kgBW/day, or placebo. The combination of quercetin and glibenclamide protected cardiomyocytes better against damage in type 2 diabetic rats than quercetin alone.

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