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Original article

# Quercetin ameliorates oxidative stress, inflammation and apoptosis in the heart of streptozotocin-nicotinamide-induced adult male diabetic rats

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ABSTRACT

*Introduction:* Quercetin is known to possess beneficial effects in ameliorating diabetic complications, however the mechanisms underlying cardioprotective effect of this compound in diabetes is not fully revealed. In this study, quercetin effect on oxidative stress, inflammation and apoptosis in the heart in diabetes were investigated. Normal and streptozotocin-nicotinamide induced adult male diabetic rats received quercetin (10, 25 and 50 mg/kg/bw) orally for 28 days were anesthetized and hemodynamic parameters i.e. systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were measured. Blood was collected for analyses of fasting glucose (FBG), insulin and cardiac injury marker levels (troponin-C, CK-MB and LDH). Following sacrificed, heart was harvested and histopathological changes were observed. Heart was subjected for analyses of oxidative stress marker i.e. lipid peroxidation and activity and expression levels of anti-oxidative enzymes i.e. SOD, CAT and GPx. Levels of inflammation in the heart were determined by measuring nuclear factor (p65-NF-κB), tumor necrosis factor (TNF-α), interleukins (IL)-1β and IL-6 levels by using enzyme-linked immunoassay (ELISA). Distribution and expression levels of TNF-α and Ikk-β (inflammatory markers), caspase-3, caspase-9, Blc-2 and Bax (apoptosis markers) in the heart were identified by immunohistochemistry and Western blotting respectively.
*Results:* Administration of quercetin to diabetic rats caused significant decrease in FBG and cardiac injury marker levels with increased in insulin levels. In diabetic rat heart, lesser histopathological changes were observed with oxidative stress, inflammation and apoptosis levels markedly decreased.
*Conclusions:* Quercetin could potentially be used to ameliorate myocardial damage due to oxidative stress, inflammation and apoptosis in diabetes.



## 1. Introduction

Diabetic cardiomyopathy (DCM) is one of the major complications of diabetes mellitus (DM). DCM is characterized by left ventricular dysfunction in the absence of underlying coronary artery disease and/or systemic arterial hypertension [1]. DCM may occur in both type 1 and 2 diabetes [1,2]. The pathophysiology underlying DCM is multifactorial which include myocardial fibrosis, myocyte hypertrophy, contractile dysfunctions, abnormal calcium handling and mitochondrial function, impaired nitric oxide signalling, and abnormal cardiomyocyte loss by apoptosis [3,4]. Despite all these, oxidative stress, which in diabetes can be induced by hyperglycemia, hyperlipidemia, and inflammation [5], is considered as the major cause for DCM [6]. In DCM, mitochondrial dysfunction could lead to over-production of reactive oxygen species (ROS), ultimately causes accumulation of advanced glycated end-(AGE) in the heart, which contributes towards accumulation of extracellular matrix (ECM) that can cause diastolic dysfunction and eventually result in heart failure [7].

Several bioactive, plant-derived compounds have been shown to have beneficial effects in ameliorating the pathogenesis of DCM. Rutin, a bioflavonoids derived from fruits such as oranges, grapes, lemons, limes, peaches, and berries has been shown to attenuate oxidative stress, apoptosis, and inflammation in the heart of diabetic rats [8]. Genistein, a compound derived from soy, has been shown to protect against cardiac inflammation and oxidative stress in streptozotocin (STZ)-induced diabetic rats [9]. There were evidences that quercetin, a naturally occurring flavonoid, that is widely present in fruits such as apples, citrus fruits, berries and green leafy vegetables may confer cardioprotection in diabetes

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[10]. Broccoli sprout extract (BSE), which contains polyphenols including quercetin, morine, genistein, luteoline and sinapic acid [11], has been shown to prevent diabetes-induced cardiac dysfunction, hypertrophy and fibrosis [12]. Quercetin has been reported to protect the heart against doxorubicin-induced cardiomyocyte toxicity [13] and experimental autoimmune myocarditis [14]. There were also reports which indicate that quercetin protects the heart against ischemic injury in normal [15] and hyperlipidemic rats [16]. Recent study by Bartekova [17] showed that administration of 15 mg/kg/day quercetin for three weeks to saline- and doxorubicin-treated adult Wistar rats enhanced function of isolated hearts through increased recovery of heart contractile function following ischemia/reperfusion. Based on these findings, we hypothesized that quercetin was able to ameliorate the progression of DCM via ameliorating cardiac oxidative stress, inflammation and apoptosis. Therefore, in this study, effects of oral quercetin consumption on hemodynamic parameters, heart histopathology, cardiac injury marker, oxidative stress, inflammation and apoptosis levels in DM were investigated.

## 2. Material and methods

### 2.1. Animals

All procedures involving animals were approved by Animal Care and Use Committee, University of Malaya and were in accordance with ARRIVE guidelines (Animal in Research-Reporting *In-Vivo* experiments) and European Community Guidelines/EEC Directive, 1986. Male SD rats aged 12 weeks and weighed 180 ± 20 g, obtained from local source were caged and maintained under standardized laboratory conditions (temperature 25 °C, 12 h light/dark cycle and 40–50% humidity). Animals were fed with standard lab food (Harlan, Rossdoff, Germany) and tap water *ad libitum*.

### 2.2. Drugs and chemicals

Streptozotocin, nicotinamide and thiobarbituric acid (TBA) were purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA). Other chemicals were of analytical grade. Creatine kinase-isoenzyme (CK-MB), troponin-I and lactate dehydrogenase (LDH) kits were purchased from Biosystems S.A., Costa Brava 30, Barcelona, Spain. ELISA kits for TNF-α, NF-kβ, IL-1β and IL-6 were purchased from Abcam Inc., Cambridge, MA 02139-1517, USA.

### 2.3. Induction of diabetes mellitus (DM) in rats

DM was induced via a single intraperitoneal (i.p) injection of nicotinamide (110 mg/kg/body weight) dissolved in normal saline 15 min prior to streptozotocin (STZ) (55 mg/kg/body weight) injection, which was dissolved in a freshly prepared 0.1 M citrate buffer (pH 4.5). These injections were given following an overnight fast [18]. Control rats (n = 6) were injected with the same amount of solvent. In order to prevent hypoglycemia in the first 24 h following STZ injection, rats were allowed to have free access to water with 5% dextrose (D5W). Three days after STZ-nicotinamide injection, rats with FBG levels greater than 7.0 mmol/L were considered as diabetic.

### 2.4. Experimental design and study protocol

Animals were divided into nine (9) groups with six (6) rats in each group:

Group I: NC Normal rats receiving sodium carboxymethyl cellulose (Na-CMC) for 28 days

Group 2: NC+10Q Normal rats receiving 10 mg/kg quercetin for 28 days

Group 3: NC+25Q Normal rats receiving 25 mg/kg quercetin for 28 days

Group 4: NC+50Q Normal rats receiving 50 mg/kg quercetin for 28 days

Group 5: DC STZ and nicotinamide-induced diabetic rats receiving Na-CMC for 28 days

Group 6: DC+10Q STZ and nicotinamide-induced diabetic rats receiving 10 mg/kg quercetin for 28 days

Group 7: DC+25Q STZ and nicotinamide-induced diabetic rats receiving 25 mg/kg quercetin for 28 days

Group 8: DC+50Q STZ and nicotinamide-induced diabetic rats receiving 50 mg/kg quercetin for 28 days

Group 9: DC+G STZ and nicotinamide-induced diabetic rats receiving 600 μg/kg glibenclamide for 28 days [19].

The selection of quercetin doses were made based on the previous reports that showed these doses confer effective cardioprotection in rats [15,20].

### 2.5. Measurement of body weight and plasma FBG levels

Body weight of the rats was determined at day 0 and day 28th. The blood was prick from tail vein on day 28th. Fasting blood glucose (FBG) was measured by using a digital glucometer (Accu-Chek1, Roche, Mannheim, Germany).

### 2.6. Hemodynamic measurements

At the end of the experimental period, animals were anaesthetized with ketamine:xylazine (80:8 mg/kg, i.p.) and tracheotomy was performed to facilitate breathing. A polyethylene catheter (PE50) filled with heparinised saline (50 IU/ml) and connected to a pressure transducer was inserted into the carotid artery. The signal was amplified by means of a bioamplifier and monitored using the PowerLab system (ADInstrument). The measurement of systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MAP) and heart rate (HR) were then obtained.

### 2.7. Measurement of serum cardiac injury marker and insulin levels

After hemodynamic measurements, the blood samples were collected from the carotid artery and serums were prepared by centrifugation of the blood samples at 2000*g* for 20 min at 4 °C. Serum levels of cardiac injury markers i.e. creatine kinase-isoenzyme (CK-MB), troponin-I and lactate dehydrogenase (LDH) were quantified by using commercially available kits. Meanwhile, serum insulin levels were determined by using enzyme-linked immunosorbent assay (ELISA) kit (EIA-2048, 96 wells, DRG Instruments GmbH, Marburg, Germany). Following blood collections, rats were humanely sacrificed and heart was collected, weighed and stored for histological, immunohistochemical, molecular biological and biochemical analyses. Cardiac somatic index was determined from the following formula:

$$\text{Cardiac somatic index} = \frac{\text{Heart weight(g)}}{\text{Body weight(g)}} \times 100$$

### 2.8. Preparation of heart homogenates

Following harvest, blood was washed off from the heart chambers by using ice-chilled physiological saline. Heart was then thawed, placed in 10% tissue homogenate buffer which was prepared using a phosphate buffer (0.1 M, pH 7.4). The homogenates were centrifuged at 600 × *g* for 10 min to remove cell debris. The supernatant was again centrifuged at 10,000 × *g* for 30 min to