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Original article

6-Gingerol Protects Against Hypertrophic Cardiomyopathy in High-Fat Diet/Low-Dose Streptozotocin-Induced Diabetic Rats

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Abstract

Background and objectives: Diabetes mellitus (DM)-induced hypertrophic cardiomyopathy (HCM) is the main reason for the high morbidity rate. 6-Gingerol has been shown to alleviate HCM by reducing oxidative stress and inflammation. The present study investigated its underlying mechanism, focusing on the activation of the AMPK/PGC-1α/SIRT1 pathway, aiming to reveal its therapeutic potential. **Methods:** Male Sprague-Dawley rats were given a high-fat diet and high fructose drink for 16 weeks and a low dose single injection of streptozotocin (22 mg/kg i.p) for DM induction. At week 8, diabetic rats were given 6-gingerol at doses of 50, 100, and 200 mg/kgBW/day for 8 weeks. Histological analysis was applied to assess cardiac structure. Western blotting was applied to assess the protein expression of AMPK/PGC-1α/SIRT1 and ELISA was used to assess the insulin signaling pathways, glutathione peroxidase (GPx), tumor necrosis factor (TNF)-α, and cardiac troponin I (cTnI). Malondialdehyde (MDA) and creatine kinase MB (CK-MB) levels were checked using colorimetric method. **Results:** We showed that 6-gingerol at the dose of 200 mg/kg/day alleviated HCM in diabetic rats and also enhanced the protein expression of AMPK/PGC-1α/SIRT1 and insulin signaling pathways while it decreased the levels of cTnI and CK-MB. It also increased antioxidative capacity proved by increased activity of GPx and decreased MDA and TNF-α levels. **Conclusion:** These findings suggest that giving 6-gingerol to the rats in doses of 50, 100, and 200 mg/kg/day may reduce DM-induced HCM, partly by activating the AMPK/PGC-1α/SIRT1 and insulin signaling pathways.

Keywords: cardiomyopathy; ginger; glucose intolerance; high-fat diets; insulin receptor substrate proteins

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Introduction

Hypertrophic cardiomyopathy (HCM) is a myocardial complication that is specific to diabetes and is distinguished by left ventricular hypertrophy, diastolic dysfunction, and eventual

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systolic impairment, regardless of the presence of either coronary artery disease or hypertension [1]. Previous research has shown that individuals with HCM and type 2 diabetes mellitus (T2DM) are at a heightened risk of poor outcomes, including progression to end-stage renal disease, stroke, heart failure, and cardiovascular mortality [2,3]. Persistent hyperglycemia and insulin resistance in type 2 diabetes disrupt cellular signaling pathways that regulate glucose uptake, metabolism, and cardiomyocyte growth, contributing to its pathological remodeling [4,5]. Although the etiology of the condition is inadequately understood, the pathophysiology of HCM is believed to be multifactorial.

The insulin signaling cascade is fundamental to these processes, involving critical mediators such as insulin receptor substrate-1 (IRS-1), phosphoinositide 3-kinase (PI3K), protein kinase B (Akt), and glucose transporter 4 (GLUT4). Disruption of this pathway results in diminished myocardial glucose consumption and aggravates cardiac hypertrophy and fibrosis [5]. The AMP-activated protein kinase (AMPK)/peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α)/sirtuin1 (SIRT1) pathway functions as a cellular energy sensor and metabolic regulator. Its activation augments insulin sensitivity, promotes glucose uptake, and suppresses pro-hypertrophic signaling [6-8].

A sedentary lifestyle along with high consumption of a high-fat, high-carbohydrate diet causes obesity, insulin resistance, and T2DM which can ultimately lead to HCM [9]. Mechanistically, high-fat and high-carbohydrate trigger oxidative stress and low-grade inflammation, as evidenced by elevated malondialdehyde (MDA), increased TNF-α, and reduced glutathione peroxidase (GPx) activity [10,11]. These changes promote cardiomyocyte hypertrophy and extracellular matrix deposition.

Despite the availability of numerous conventional medications for T2DM, the adverse effects they induce occasionally lead patients to discontinue their therapy. Consequently, there is a necessity for pharmaceuticals derived from natural substances that are equally efficacious as conventional medications but exhibit more bearable adverse effects. Recent epidemiological research indicates that extracts from herbs, vegetables, fruits, and spices can diminish the risk of cardiovascular diseases [12,13]. 6-Gingerol is a phenolic compound that is isolated from ginger. It is an important part of gingerols and has significant antioxidant and anti-inflammatory activities [13]. Recently, we have also reported that 6-gingerol at doses of 50, 100, and 200 mg/kg/day in rats with metabolic syndrome was able to improve insulin sensitivity and reduce lipid accumulation in the liver and adipose tissue [14]. Furthermore, a previous study indicated that 6-gingerol can reduce myocardial ischemia/reperfusion injury by inhibiting apoptosis and activating the PI3K/AKT pathway [15]. Peng et al. recently reported that 6-gingerol at a dose of 12.5 mg/kg/day in mice subjected to a high-fat diet improved lipid metabolism abnormalities in skeletal muscle via enhancing AMPK/sirtuin-1 (SIRT-1)/peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) activity [16]. While prior studies have shown its protective effects in metabolic and cardiovascular disorders, its direct action on AMPK/PGC-1α/SIRT1 activation and insulin signaling restoration in diabetic hearts has not been fully elucidated. This study aimed to investigate whether 6-gingerol can attenuate cardiac hypertrophy in diet-induced diabetic rats by modulating oxidative stress and activating AMPK/PGC-1α/SIRT-1, alongside restoration of insulin signaling. The findings are expected to provide novel insight into the mechanistic basis for cardioprotective effects of 6-gingerol in HCM.

Material and Methods

Ethical considerations

The animal study protocol was approved by the Institutional Animal Care and Use Committee of the Faculty of Medicine, Universitas Indonesia (KET-945/UN2.F1/ETIK/PPM.00.02/2021). The guideline for handling laboratory animals includes the 3Rs principle (Replacement, Reduction, Refinement), ethical approval, species-appropriate care and welfare, low-stress handling techniques, detailed procedural documentation, and personnel training to ensure ethical and standardized treatment.

Chemicals

The high-fat diet (HFD) was from Brawijaya University, East Java, Indonesia; corn oil was from Mazola Ltd. (Indonesia); 6-gingerol was from Actin chemicals, China (batch #23153-14-6, and ≥98% purity). Streptozotocin was purchased from Santa Cruz, USA (catalog #sc-200719A). Primary antibodies against AMPKα, phospho-

AMPKα, and GAPDH and secondary antibody horseradish peroxidase (HRP)-conjugated anti-rabbit IgG were obtained from Cell Signaling Technology, Inc. (USA) with catalog number of #CST-2532, #CST-2535, #CST-5174S, and #CST-7074, respectively. Primary antibodies against PGC-1α and SIRT-1 were purchased from Santa Cruz Biotechnology, Inc. (USA) with catalog number of #sc-518025 and sc-135792, respectively. Cardiac troponin-I ELISA kit (cTn-I; catalog number of #CSB-E08594r) was purchased from the Cusabio, USA. Glucose transporter-4 (GLUT-4; catalog number of #E-EL-R0430), insulin receptor substrate-1 (IRS-1; catalog number of #E-EL-R1111), and insulin (INS; catalog number of #E-EL-R3034) ELISA kits were purchased from the Elabscience Bionovation, Inc., USA. Phosphoinositide 3-kinases (PI-3K; catalog number of #MBS260381) and glutathione peroxidase (GPx; catalog number of #MBS1600242) ELISA kits were obtained from the MyBiosource, Inc., USA. Protein kinase B (PKB or Akt; catalog number of #ER1268) ELISA kit was obtained from the FineTest, China. Tumor necrosis factor-α (TNF-α; catalog number of BZ-08184670-EB) ELISA kit was obtained from Bioenzy, USA. Malondialdehyde (MDA) was measured using lipid peroxidation assay kit from Sigma-Aldrich (catalog number of #MAK568). All other chemicals and reagents were analytical reagent grade and purchased from the Sigma-Aldrich, Germany.

Experimental animals

A total of 30 male Sprague-Dawley rats, weighing 180-220 g, were obtained from the National Agency of Drug and Food Control, Jakarta, Indonesia. All rats were housed in standard polypropylene plastic cages at a controlled temperature (24-26 °C) and humidity (65%-75%) with a 12 h light/dark cycle. Rats had free access to food and water. After one week of acclimatization to the laboratory conditions, the rats were randomly divided into 5 groups, namely normal-control (C), diabetes mellitus (DM), and combination of both DM plus 6-gingerol (6-G) at doses of 50 mg/kg/day (DM6-G 50), 100 mg/kg/day (DM6-G 100), and 200 mg/kg/day (DM6-G 200), with 6 rats in each group. The selective dosage of 6-gingerol used in this study was derived from a previous study [14]. 6-Gingerol was dissolved in 1.5 mL/kg of corn oil and given once daily by gavage for 8 weeks. Rats in the C group received standard pellet diet, while rats in the DM groups received high-fat diet (HFD). The composition of experimental diets was as follows: the standard diet contained 4% fat, 20% protein, 47% carbohydrate, 12% water, and 17% minerals, with a total of 304 kcal/100 g, whereas HFD contained 29.02% fat, 21.31% protein, 31.92% carbohydrate, 12.60% water, and 5.15% minerals, with a total of 474 kcal/100 g. Fifty-five percent of fructose was administered by gavage (2 times daily, 1.5 mL/day) to the rats receiving HFD and at week 8, a single dose of streptozotocin (STZ) (22 mg/kg) dissolved in fresh sodium citrate buffer pH 4.5 was injected intraperitoneally. All diets and fructose drinks were given throughout the study period of 16 weeks. Fifty-five percent of fructose was given with the aim of establishment and progression of liver fat deposition to fibrogenesis and insulin resistance. In the 8th-week, after confirming that the rats subjected to a HFD, fructose consumption, and STZ injections developed DM, indicated by fasting blood glucose (FBG) levels of ≥ 250 mg/dL, they were subsequently randomized to receive graded dosages of 6-gingerol [14]. At the end of the study, all rats were sacrificed by administering a high dose of anesthesia, namely a single i.p. injection of ketamine/xylazine (87 mg/kg and 13 mg/kg) and the blood samples were collected into a sterile tube by cardiac puncture. Serum was collected by centrifugation at 2000 rpm for 10 min at 4 °C and then stored at –20 °C for further analysis. The heart tissues were isolated from rats, immediately washed with ice cold saline, weighed, and excised, and the heart weight (HW)-to-body weight (BW) ratio was calculated, and thereafter the heart tissues were kept on an Eppendorf tube until analysis. One part of the heart tissue was stored at –80 °C for biochemical assays, and the remaining was fixed in 4% formaldehyde for histopathological analysis.

Determination of glycemia, blood pressure, and cardiac markers

Fasting serum glucose levels were measured using Autocheck® Glucare glucometer (Medical Technology Promedr, St. Ingbert, Germany). Serum insulin was measured using ELISA kits. Homeostasis model assessment for insulin resistance (HOMA-IR) were calculated by applying the formula: fasting serum glucose (mg/dL) x fasting serum insulin (IU/mL)/405.

Systolic (SBP) and diastolic blood pressure (DBP) were measured three times using the tail-cuff method. In brief, the rats were placed in individual

restrainers and acclimatized in a quiet, warm environment for 10-15 minutes before measurement. An inflatable cuff with a pressure sensor was placed around the tail. The cuff was inflated to occlude blood flow and then gradually deflated while the return of blood flow was detected by the sensor. Systolic and diastolic blood pressure were recorded automatically, and the average of at least three consecutive, consistent readings was used for analysis. Data were presented as mean arterial pressure (MAP) by applying the formula: DBP+1/3 (SBP – DBP). The serum levels of creatine kinase-MB (CK-MB) was detected with a colorimetric method and the absorbance of the colored solution was measured at 340 nm, whereas serum levels of cTn-I were measured using ELISA kit [17].

Determination of malondialdehyde, TNF-α, and levels of glutathione peroxidase

The myocardial tissue samples (100 mg) were homogenized in physiological NaCl solution (10% W/V) using an Ultra Turrax,® then centrifuged at 3000 *rpm*, 4 °C for 10 min. Biochemical analysis was performed using the supernatant. Levels of GPx and TNF-α were measured using ELISA kits. Malondialdehyde (MDA) levels were measured using a lipid peroxidation assay kit, following the manufacturer's instructions. Absorbance was read using a spectrophotometer at a wavelength of 530 nm, and MDA concentrations were calculated based on a standard curve.

Determination of IRS-1, PI3K, Akt, and GLUT-4 protein levels in myocardial tissues

IRS-1, PI3K, Akt, and GLUT-4 protein levels in myocardial tissue were detected by ELISA kits according to the manufacturer's instructions. In brief, myocardial tissues were collected, weighed to 100 grams, rinsed with cold phosphate-buffered saline (PBS) to remove blood, and homogenized in an appropriate volume of ice-cold lysis buffer provided by the manufacturers. The homogenate was subsequently centrifuged at 10,000 x *g* for 10-15 min at 4 °C to obtain the clear supernatant. The supernatant was collected and used immediately for ELISA analysis.

Western blotting for AMPK, PGC-1α, and SIRT-1 protein expression in myocardial tissues

Myocardial tissue (100 mg) was cut into small pieces using clean scissors and put at the homogenizer. The tissue was homogenized in an ice-cold Tris buffer containing 50 mM Tris-HCl (pH 7.4), 200 mM NaCl, 20 mM NaF, 1 mM Na_3VO_4, 1 mM 2-mercaptoethanol, 0.01 mg/mL leupeptin, 0.01 mg/mL aprotinin. Samples were centrifuged at 3,000 rpm, 4 °C for 10 min, and supernatant were collected and stored at −80 °C until analysis. The bicinchoninic acid (BCA) technique was used to assess the sample's total protein content. To determine the protein expression levels of AMPK, phospho-AMPK, PGC-1α, and SIRT-1, equal amounts of protein (50 μg) extracts were separated by SDS-PAGE (Bio-Rad, USA) and transferred electrophoretically to nitrocellulose membranes. The membranes were then blocked with 5% non-fat dried milk (for PGC-1α and SIRT-1) or 5% bovine serum albumin (for AMPK and phospho-AMPK) in Tris-buffered saline Tween (20 mM Tris (pH 7.6), 137 mM NaCl, and 0.1% Tween-20). All of the antibodies were used at a dilution of 1:1000. The membrane was then incubated overnight at 4 °C with the primary antibody, and the bound antibody was visualized using horse-radish peroxidase-conjugated secondary antibody and chemiluminescence developing agents (Amersham Biosciences, UK). The level of GAPDH was estimated in every sample to ensure equal protein loading of the samples. The band densities were quantified with densitometric analysis using ImageJ.

Evaluation of histopathology

To evaluate myocardial hypertrophy, we performed morphological analysis of myocardial sections. In brief, the excised myocardial tissues were cut into 5-μm-thick transverse slices and fixed in 10% formalin, followed by embedding in paraffin according to standard histological protocols. Sections of myocardial samples from each group were stained with hematoxylin and eosin (H&E) to evaluate cardiomyocyte cross-sectional areas. ImageJ software was used to observe and calculate the cross-sectional areas of individual cardiomyocytes.

Statistical analysis

All data are presented as the means±standard error of the mean, and a one-way ANOVA test followed by a post hoc Tukey test was used to determine statistical significance. The Shapiro-Wilk test for normality was conducted to ascertain the data distribution. All analyses were performed in SPSS 23.0 statistical software. A p value< 0.05 was considered to indicate statistical significance.

Results and Discussion

This study demonstrated that 6-gingerol, mostly at doses ranging from 50 mg/kg/day to 200 mg/kg/day, significantly alleviated cardiomyocyte hypertrophy and inflammation in diabetic rats subjected to a high-fat diet and high-fructose intake over 16 weeks. Furthermore, our results indicated that 6-gingerol administered over 8 weeks could mitigate insulin resistance, evidenced by a reduction in HOMA-IR and fasting blood glucose levels, as well as decreased CK-MB and cTnI levels, alongside the inhibition of oxidative stress in myocardial tissues, as demonstrated by lowered MDA levels and elevated GPx levels. The current investigation identified elevated protein levels of p-AMPK, SIRT-1, and PGC-1α, along with enhanced insulin signaling pathways, following the administration of 6-gingerol.

It is known that long-term consumption of a high-fat diet, high-fructose consumption induces obesity, insulin resistance, chronic oxidative stress, and inflammation, which have been implicated in the pathophysiology of diabetes-associated cardiovascular disease (CVD) [5,18]. Studies have shown that insulin resistance in T2DM is initiated and progressed by oxidative stress, which damages insulin signaling system [5,10,11]. The current study demonstrated that HFD and fructose consumption for 16 weeks caused increased fasting blood glucose levels (Figure 1A), insulin resistance (Figure 1B), increased body weight (Figure 2A), and oxidative stress based on the imbalance between MDA (Figure 3A) and GPx (Figure 3B) levels as well as an increased in TNF-α levels (Figure 3C). Interestingly, we found that 6-gingerol alleviated all of those disorders. Improvements in all those parameters were especially significant at the dose of 200 mg/kg/day compared to the DM group. In line with previous reports, gingerol significantly downregulated blood glucose levels, attenuated oxidative stress, and decreased insulin resistance [19,20]. Furthermore, as illustrated in Figure 2B, the heart weight/body weight ratio in the DM group was elevated relative to the C group, indicating the development of cardiac hypertrophy, despite no significant rise in mean arterial pressure (Figure 2E). All of these parameters restored by 6-gingerol treatment, although they did not achieve statistical significance. Further studies are needed to determine whether 6-gingerol is able to reduce blood pressure.

Abnormal production of ROS is a hallmark of cardiovascular diseases. The major metabolic sensor and energy-level regulator AMPK has recently been identified as a redox sensor that aids in maintaining cardiovascular physiology and delaying the advancement of illness [21].

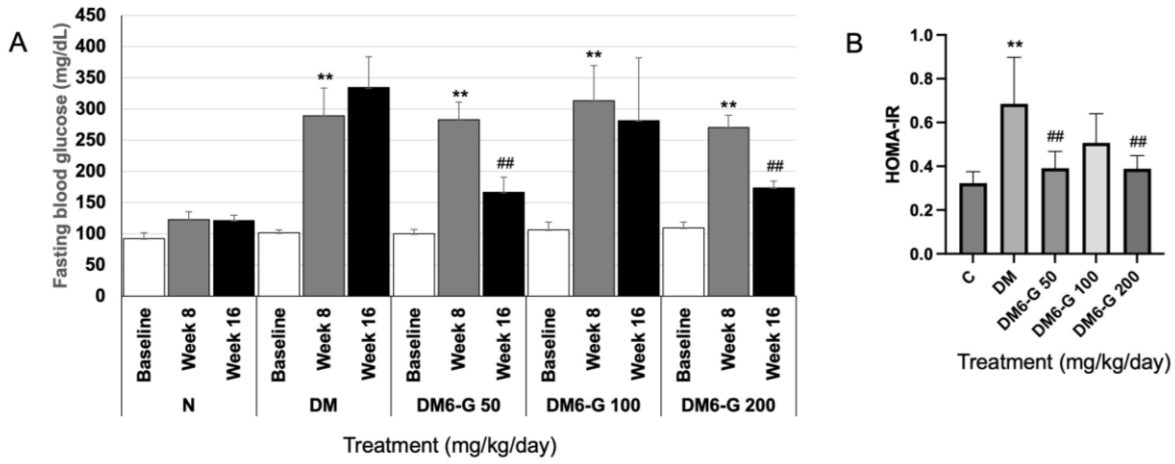

Figure 1. Suppression of fasting plasma glucose levels (A) and homeostatic model assessment for insulin resistance (HOMA-IR) (B); C: control; DM: diabetic rats; DM6-G 50, DM6-G 100 and DM6-G 200: diabetic rats treated with 6-gingerol at doses of 50, 100 and 200 mg/kg/day, respectively; the data were expressed as mean ± standard error of the mean (SEM); $^{**}p<0.01$ compared with C group, and $^{\#\#}p<0.01$ compared with DM group

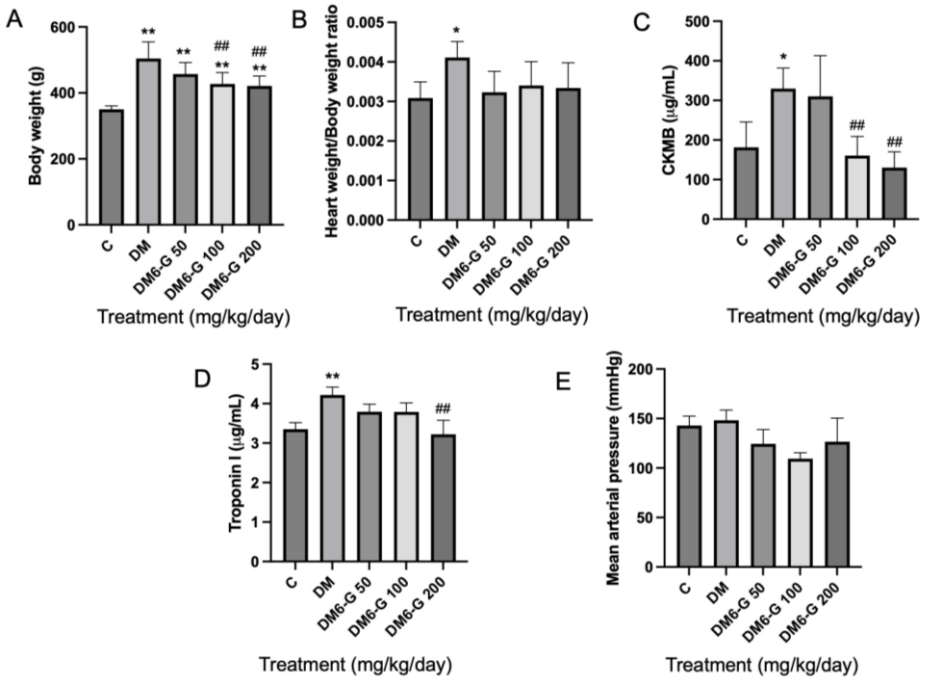

Figure 2. The effects of gingerol on body weight (A), heart weight-to-body weight ratio (B), serum levels of creatinine kinase-MB (CKMB) (C), cardiac troponin I (D), and mean arterial pressure (E) in groups; C: control; DM: diabetic rats; DM6-G 50, DM6-G 100 and DM6-G 200: diabetic rats treated with 6-gingerol at doses of 50, 100 and 200 mg/kg/day, respectively; $^{*}p<0.05$; $^{**}p<0.01$ compared with C group, and $^{\#\#}p<0.01$ compared with DM group

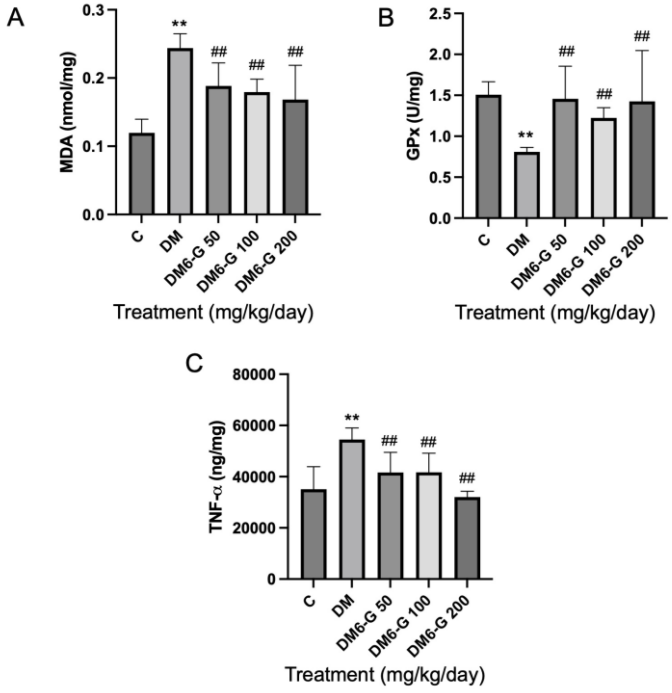

Figure 3. Effect of 6-gingerol treatment on oxidative stress and inflammation in myocardial tissues; malondialdehyde (MDA) levels (A), glutathione peroxidase (GPx), (B) and tumor necrosis factor-α (TNF-α) (C) in myocardial tissues of each group; C: control; DM: diabetic rats; DM6-G 50, DM6-G 100 and DM6-G 200: diabetic rats treated with 6-gingerol at doses of 50, 100 and 200 mg/kg/day, respectively; the data were expressed as mean ± standard error of the mean (SEM). $^{**}p<0.01$ compared with C group, and $^{\#\#}p<0.01$ compared with DM group

Moreover, AMPK and SIRT-1 are fuel-sensing molecules that share similar target molecules and regulate each other to inhibit ROS production and inflammatory cytokines [6,16,21,22]. While SIRT1 is vital in regulating a wide range of critical biological functions, such as oxidative stress, apoptosis, and autophagy, PGC-1α is abundantly expressed in cardiac myocytes and plays an important role in cardioprotective therapy by inducing gene expression of antioxidant [23-25]. Tian et al., have shown that the downstream molecules PGC-1α and SIRT1 may be influenced by AMPK, and SIRT1 may also be involved in the activation of AMPK and PGC-1α [26]. Recently, it has been reported that a high-fat high-fructose diet could lead to increase of oxidative stress and downregulation of AMPK, PGC-1α, and SIRT-1 [27]. There is evidence that the activation of AMPK is related to several hormones and natural drugs. Some natural plant products have been shown to recover high fructose-induced low levels of p-AMPK in cardiomyocytes and diabetic mice [28,29]. In addition, ginger and its bioactive compound, 6-gingerol, were able to upregulate AMPK phosphorylation, PGC-1α, and SIRT1, thereby not only preventing arsenic trioxide-induced cardiotoxicity but also obesity in mice [30,31]. Consistently, we found that 6-gingerol administration for 8 weeks at all three doses significantly decreased oxidative stress, inflammation, and increased the protein levels of the active form of AMPK (p-AMPK), PGC-1α, and SIRT-1 in rats fed high-fat and high-fructose consumption (Figure 5A-D).

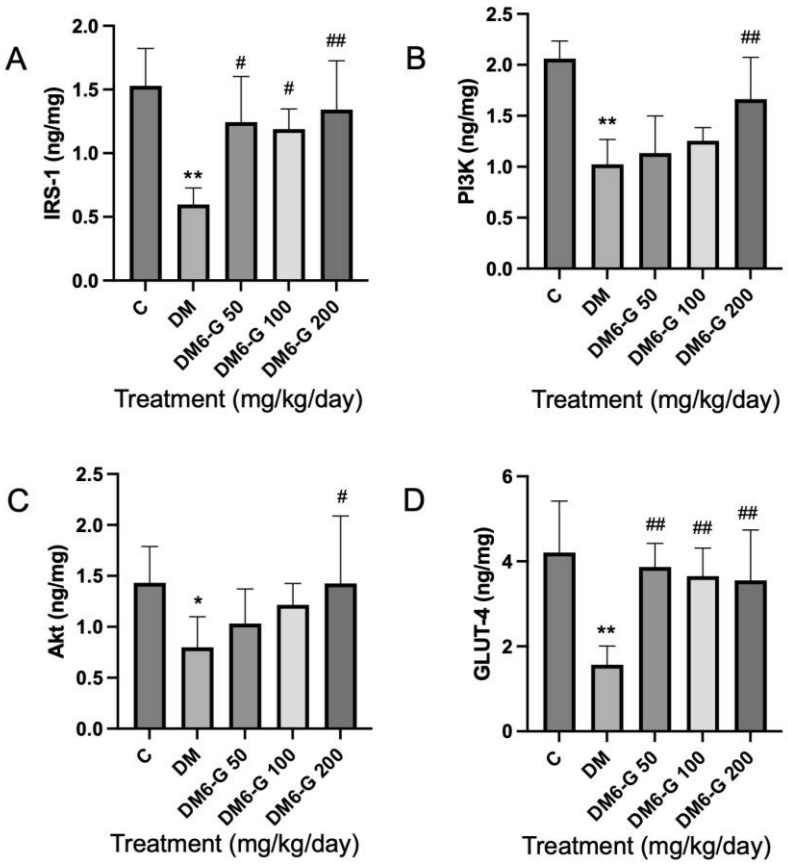

Figure 4. Effect of 6-gingerol on protein expression in myocardial tissues; IRS-1 (A), PI3K (B), Akt (C), and GLUT-4 (D); C: control; DM: diabetic rats; DM6-G 50, DM6-G 100 and DM6-G 200: diabetic rats treated with 6-gingerol at doses of 50, 100 and 200 mg/kg/day, respectively. The data were expressed as mean ± standard error of the mean (SEM). $^{*}p<0.05$, $^{**}p<0.01$ compared with C group, $^{\#}p<0.05$ and $^{\#\#}p<0.01$ compared with DM group

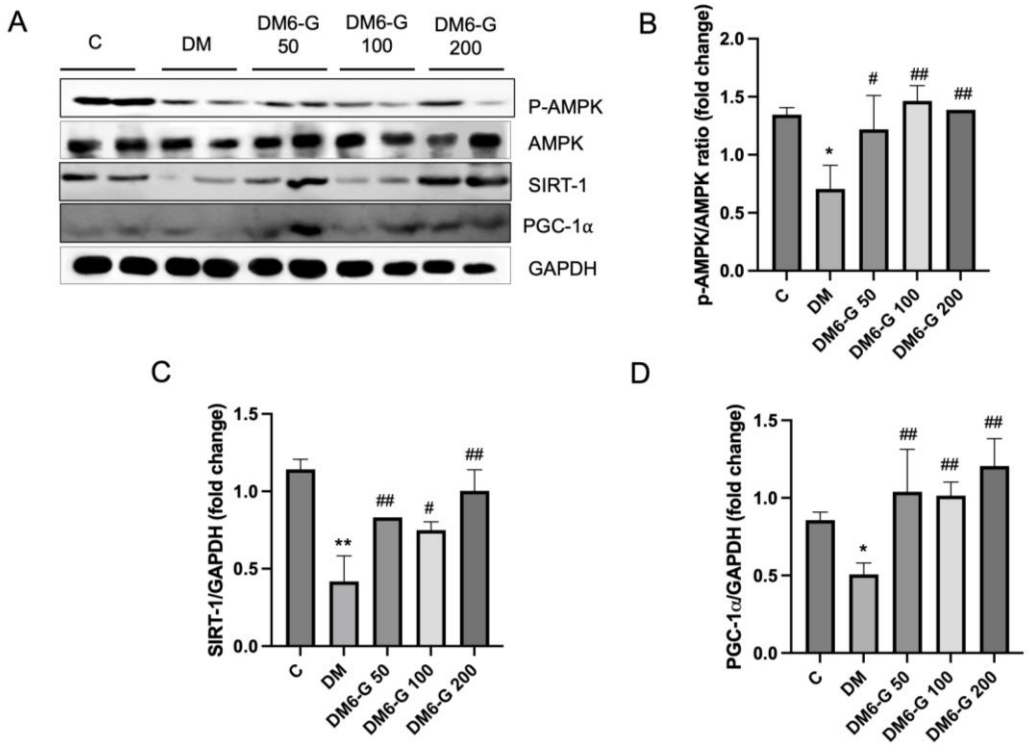

Figure 5. Effect of 6-gingerol on AMPK/SIRT-1/PGC-1α signaling; the protein expressions of p-AMPK, SIRT-1, and PGC-1α were detected in cardiac tissue by Western blotting (A); 6-gingerol increased the phosphorylation of AMPK (B) and increased the protein expression of SIRT-1 (C) and PGC-1α (D); C: control; DM: diabetic rats; DM6-G 50, DM6-G 100 and DM6-G 200: diabetic rats treated with 6-gingerol at doses of 50, 100 and 200 mg/kg/day, respectively; the data were expressed as mean ± standard error of the mean (SEM). $^{*}p<0.05$, $^{**}p<0.01$ compared with C group, $^{\#}p<0.05$ compared with DM group, and $^{\#\#}p<0.01$ compared with DM group

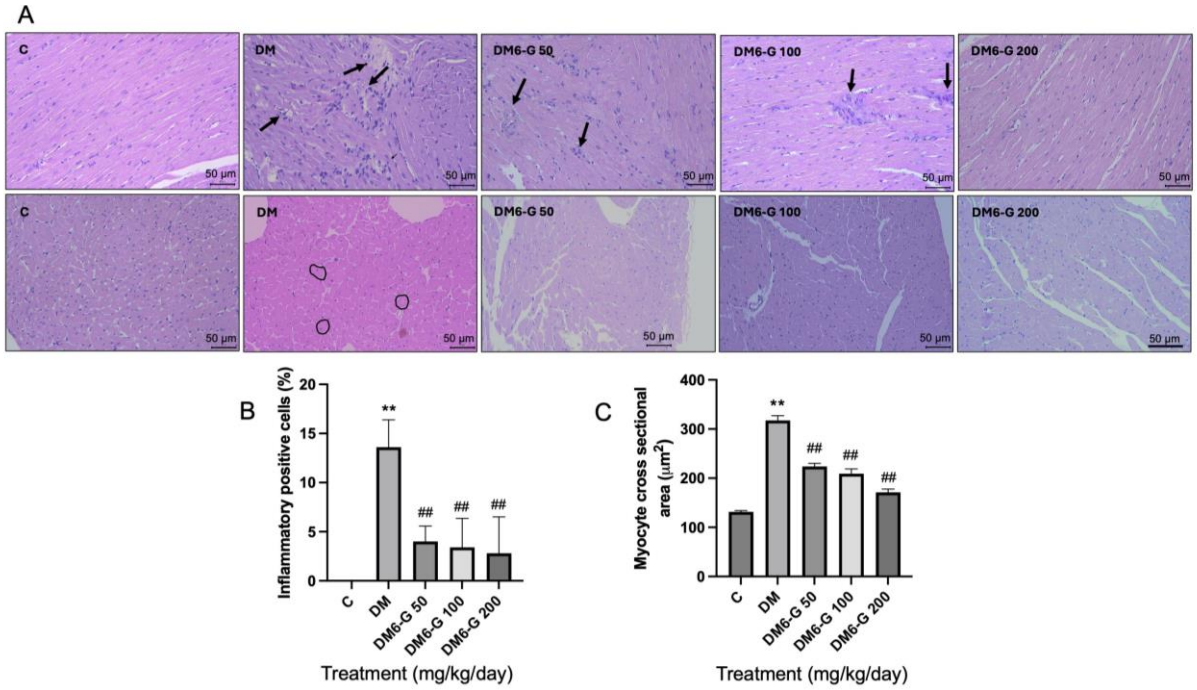

Figure 6. 6-Gingerol inhibition of myocardial inflammation and cardiomyocyte hypertrophy in diabetic rats; representative images of H&E stained samples (x400); black arrows represented inflammatory cells (A); quantitative analysis of inflammatory positive cells (B), and cross-sectional area of cardiomyocytes shown with black circumference (C); scale bar: 50 µm; C: control; DM: diabetic rats; DM6-G 50, DM6-G 100 and DM6-G 200: diabetic rats treated with 6-gingerol at doses of 50, 100 and 200 mg/kg/day, respectively; the data were expressed as mean ± standard error of the mean (SEM). $^{**}p<0.01$ compared with C group, and $^{\#\#}p<0.01$ compared with DM group; n=6

Moreover, we have shown that oxidative stress brought on by HFD and fructose consumption disrupts the insulin signaling system, which in turn causes insulin resistance. One important signaling mechanism linked to insulin resistance is the PI3K/Akt pathway, which is essential for many physiological processes, including the transfer of glucose [15]. The activation of PI3K is essential for glucose uptake, with Akt serving as the principal signaling molecule following PI3K activation [15]. GLUT4, the principal glucose transporter in cardiomyocytes, facilitates glucose uptake, thereby regulating energy metabolism and growth in the heart [32]. In addition, overexpression of PGC-1α has been shown to induce GLUT4 mRNA through the coactivation of MEF2C in L6 muscle cells [33].

The current study reveals that the protein expression levels of IRS-1, PI3K, Akt, and GLUT4 in cardiomyocytes of HFD and fructose consumption-induced insulin resistance were significantly lower than those in normal control rats. Treatment with 6-gingerol resulted in a significant upregulation of IRS-1 and GLUT4 protein expressions across all three doses (Figures 4A & 4D). The 200 mg/kg/day oral dose of 6-gingerol significantly increased the protein expression of PI3K and Akt (Figures 4B & 4C). Similar alterations in the protein levels of IRS-1, PI3K, Akt, and GLUT4 were seen in the skeletal muscle of *ob/ob* mice [34]. In the previous study, we reported that 6-gingerol (200 mg/kg/day, p.o.) increased mean α- and β-cells pancreas which correlates to glycemic control [35]. Consequently, our hypothesis was that HFD and fructose consumption-induced insulin resistance causes aberrant glucose metabolism and oxidative stress, potentially through interfering with the PI3K/Akt and AMPK signaling pathways, which translocate GLUT4 to the cell membrane for glucose uptake. Our results from the biochemical analyses are supported by histopathological findings. It was found that feeding rats an HFD and fructose consumption for 16 weeks had a detrimental effect on the heart's structural integrity. Increased cardiomyocyte diameter cells and inflammatory cells were observed in the cardiac tissue of the DM group; treatment with 6-gingerol at all three doses significantly improved these diseases (Figures 6A-C).

Conclusion

6-Gingerol at the doses of 50, 100, and 200 mg/kg/day by upregulating the expression of PI3K/Akt and AMPK/SIRT-1/PGC-1α proteins can increase GLUT4 expression, which results in reducing HOMA-IR, inflammatory processes, and increasing antioxidant enzyme levels in the heart tissue of HFD and fructose consumption-induced diabetic rats. This leads to improved cardiac structure and reduced inflammatory cells. Future investigations into the precise pharmacokinetics of 6-gingerol and the duration of use are necessary to expand our understanding. One of the notable limitations of this study is the lack of direct cardiac function analysis, such as echocardiography or hemodynamic measurements. However, the significant reduction in cTnI and CK-MB levels in the treatment group provided indirect evidence of reduced myocardial injury, supporting a potential cardioprotective role of 6-gingerol in HCM. Future studies incorporating in vivo functional assessment will be essential to fully elucidate the therapeutic potential of 6-gingerol in diabetic cardiomyopathy.

AI tools

None

Acknowledgments

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Author Contributions

Vivian Soetikno contributed to the conceptualization; Vivian Soetikno and Andika Yusuf Ramadhan contributed to the methodology; Vivian Soetikno, Andika Yusuf Ramadhan, Savira Wijaya, Diski Saisa, Syifa Nurfitriyanti, and Bryantlewi Santoso contributed to the formal analysis; Vivian Soetikno, Kusmardi Kusmardi, Shirly Gunawan, Somasundaram Arumugam, and Remya Sreedhar contributed to the validation and manuscript writing. All authors have read and agreed to the published version of the manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Abbreviations

6-G: 6-gingerol; T2DM: type 2 diabetes mellitus; HCM: hypertrophic cardiomyopathy; ROS: reactive oxygen species; STZ: streptozotocin; IRS-1: insulin receptor substrate-1; PI3K: phosphoinositide-3-kinase; Akt/PKB: protein kinase B; GLUT4: glucose transporter-4; AMPK: adenosine monophosphate (AMP)-activated protein kinase; PGC-1α: peroxisome proliferator-activated receptor γ coactivator 1-α; SIRT1: sirtuin1; MDA: malondialdehyde; TNF-α: tumor necrosis factor-α; GPx: glutathione peroxidase; FBG: fasting blood glucose; HOMA-IR: homeostatic model assessment for insulin resistance; CK-MB: creatinine kinase-MB; cTn-I: cardiac troponin I; SBP: systolic blood pressure; DBP: diastolic blood pressure