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Cardioprotective Effects of 6-Gingerol on Hypertrophic Cardiomyopathy in Diabetic Rats Induced by a High-Fat Diet and Low-Dose Streptozotocin

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ABSTRACT

Hypertrophic cardiomyopathy (HCM) associated with diabetes mellitus (DM) contributes significantly to elevated mortality rates. Previous studies have demonstrated that 6-gingerol mitigates HCM through attenuation of oxidative stress and inflammatory responses. This research explored the mechanisms involved, with particular emphasis on the AMPK/PGC-1α/SIRT1 signaling cascade, to assess its potential as a treatment option. Male Sprague-Dawley rats were fed a high-fat diet combined with high-fructose water for 16 weeks, followed by a single low-dose intraperitoneal injection of streptozotocin (22 mg/kg) to induce diabetes. Starting from week 8, the diabetic animals received oral administration of 6-gingerol at 50, 100, or 200 mg/kg body weight daily for 8 weeks. Cardiac tissue morphology was evaluated using histopathology. Protein levels of AMPK/PGC-1α/SIRT1 were measured via Western blot analysis, while ELISA quantified components of insulin signaling, glutathione peroxidase (GPx) activity, tumor necrosis factor-α (TNF-α), and cardiac troponin I (cTnI). Colorimetric assays determined malondialdehyde (MDA) and creatine kinase MB (CK-MB) concentrations. Administration of 6-gingerol at 200 mg/kg/day effectively reduced HCM features in diabetic rats, increased expression of AMPK/PGC-1α/SIRT1 proteins and insulin signaling components, and lowered cTnI and CK-MB concentrations. Additionally, it improved antioxidant defense by elevating GPx activity and reducing MDA and TNF-α levels. The results indicate that daily oral doses of 6-gingerol (50, 100, and 200 mg/kg) can mitigate diabetes-related HCM, potentially through enhancement of the AMPK/PGC-1α/SIRT1 axis and restoration of insulin signaling pathways.

Keywords: Cardiomyopathy, Ginger, Glucose intolerance, High-fat diets, Insulin receptor substrate proteins

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Introduction

Diabetic hypertrophic cardiomyopathy (HCM) represents a distinct cardiac complication of diabetes, characterized by left ventricular thickening, impaired relaxation, and progressive contractile dysfunction, independent of coronary disease or hypertension [1]. Studies have indicated that patients with type 2 diabetes mellitus (T2DM) and HCM face increased risks of adverse events, such as advanced kidney failure, cerebrovascular incidents, congestive heart failure, and death from cardiovascular causes [2, 3]. Chronic high blood glucose and resistance to insulin in T2DM interfere with key pathways controlling sugar utilization, energy metabolism, and heart muscle cell proliferation, leading to structural changes in the myocardium [4, 5].

The development of HCM involves multiple factors, though its precise causes remain unclear. Central to this is the insulin pathway, which includes essential elements like insulin receptor substrate-1 (IRS-1), phosphoinositide 3-kinase (PI3K), protein kinase B (Akt), and glucose transporter 4 (GLUT4). Impairments here lead to reduced cardiac glucose use and worsened hypertrophy and scarring [5]. The AMP-activated protein kinase (AMPK)/peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α)/sirtuin1 (SIRT1)

system acts as an important regulator of cellular energy balance and metabolism. When activated, it improves insulin responsiveness, facilitates glucose transport, and inhibits signals promoting hypertrophy [6-8].

Unhealthy habits, including lack of physical activity and excessive intake of fatty and sugary foods, promote weight gain, insulin resistance, and T2DM, eventually resulting in HCM [9]. These dietary patterns induce oxidative damage and chronic mild inflammation, shown by higher MDA, elevated TNF-α, and lower GPx function [10, 11]. Such alterations encourage excessive growth of heart muscle cells and buildup of connective tissue. Although many standard drugs exist for managing T2DM, their side effects sometimes cause patients to stop treatment. This highlights the need for naturally sourced alternatives that offer similar benefits with fewer tolerability issues.

Evidence from population studies suggests that compounds from plants, produce, and seasonings may lower the likelihood of heart-related illnesses [12, 13]. 6-Gingerol, a key bioactive phenol derived from ginger, stands out among gingerols for its strong antioxidant and anti-inflammatory properties [13]. In prior work, we found that 6-gingerol administered at 50, 100, and 200 mg/kg/day in rats with metabolic syndrome enhanced insulin responsiveness and decreased fat buildup in liver and fat tissues [14]. Another investigation showed that 6-gingerol protects against heart damage from ischemia/reperfusion by preventing cell death and stimulating the PI3K/AKT route [15]. Recent findings by Peng *et al*. indicated that 6-gingerol at 12.5 mg/kg/day in high-fat-fed mice corrected lipid issues in muscle tissue by boosting AMPK/sirtuin-1 (SIRT-1)/peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) function [16].

Although earlier research has highlighted its benefits in metabolic and heart conditions, the specific role of 6-gingerol in stimulating AMPK/PGC-1α/SIRT1 and repairing insulin pathways in diabetic cardiac tissue requires further clarification. The goal of this investigation was to determine if 6-gingerol could lessen heart muscle thickening in rats with diet-induced diabetes by influencing oxidative stress, activating AMPK/PGC-1α/SIRT-1, and improving insulin signaling. These insights may offer new understanding of how 6-gingerol provides heart protection in HCM.

Materials and Methods

Ethical considerations

The protocol for animal experiments received approval from the Institutional Animal Care and Use Committee at the Faculty of Medicine, Universitas Indonesia (KET-945/UN2.F1/ETIK/PPM.00.02/2021). Animal care adhered to the 3Rs principles (Replacement, Reduction, Refinement), including ethical review, suitable species management, welfare standards, gentle handling methods, thorough procedure records, and staff training for consistent and humane practices.

Chemicals

The high-fat diet (HFD) originated from Brawijaya University, East Java, Indonesia; corn oil came from Mazola Ltd. (Indonesia); 6-gingerol was sourced from Actin chemicals, China (batch #23153-14-6, ≥98% purity). Streptozotocin was acquired from Santa Cruz, USA (catalog #sc-200719A). Primary antibodies targeting AMPKα, phospho-AMPKα, and GAPDH, along with HRP-conjugated anti-rabbit IgG secondary antibody, were from Cell Signaling Technology, Inc. (USA) (catalog numbers #CST-2532, #CST-2535, #CST-5174S, and #CST-7074, respectively). Antibodies for PGC-1α and SIRT-1 were obtained from Santa Cruz Biotechnology, Inc. (USA) (catalog numbers #sc-518025 and sc-135792, respectively). The cardiac troponin-I ELISA kit (cTn-I; catalog #CSB-E08594r) was from Cusabio, USA. ELISA kits for glucose transporter-4 (GLUT-4; #E-EL-R0430), insulin receptor substrate-1 (IRS-1; #E-EL-R1111), and insulin (INS; #E-EL-R3034) were from Elabscience Bionovation, Inc., USA. Kits for phosphoinositide 3-kinases (PI-3K; #MBS260381) and glutathione peroxidase (GPx; #MBS1600242) were supplied by MyBiosource, Inc., USA. The protein kinase B (PKB or Akt; #ER1268) ELISA kit was from FineTest, China. The tumor necrosis factor-α (TNF-α; #BZ-08184670-EB) ELISA kit was from Bioenzy, USA. Malondialdehyde (MDA) assessment used a lipid peroxidation kit from Sigma-Aldrich (catalog #MAK568). Remaining chemicals and reagents were of analytical grade from Sigma-Aldrich, Germany.

Experimental animals

Thirty male Sprague-Dawley rats, initially weighing between 180 and 220 g, were sourced from the National Agency of Drug and Food Control in Jakarta, Indonesia. The animals were maintained in conventional

polypropylene cages under regulated environmental conditions, including a temperature range of 24–26°C, relative humidity of 65–75%, and a 12-hour light/dark cycle. Food and water were provided ad libitum. Following a 7-day adaptation period to the facility, the rats were allocated randomly into five groups of six animals each: a normal control group (C), a diabetes mellitus group (DM), and three diabetic groups treated with 6-gingerol (6-G) at daily doses of 50 mg/kg (DM6-G 50), 100 mg/kg (DM6-G 100), and 200 mg/kg (DM6-G 200). The chosen doses of 6-gingerol were based on prior research [14]. The compound was suspended in corn oil at a volume of 1.5 mL/kg and administered orally via gavage once daily for 8 weeks. Animals in the control group were fed a standard pellet diet, whereas those in the diabetic groups received a high-fat diet (HFD). The standard diet consisted of 4% fat, 20% protein, 47% carbohydrate, 12% water, and 17% minerals, providing 304 kcal/100 g. In contrast, the HFD comprised 29.02% fat, 21.31% protein, 31.92% carbohydrate, 12.60% water, and 5.15% minerals, yielding 474 kcal/100 g. Rats assigned to the HFD regimen also received 55% fructose solution via gavage (twice daily, 1.5 mL/day). At week 8, diabetes was induced with a single intraperitoneal injection of streptozotocin (STZ) at 22 mg/kg, freshly prepared in sodium citrate buffer (pH 4.5). Both the specialized diets and fructose administration continued throughout the 16-week experimental duration. The high-concentration fructose was intended to promote hepatic fat accumulation, progression to fibrogenesis, and development of insulin resistance. Confirmation of diabetes onset in the targeted groups occurred at week 8, defined by fasting blood glucose levels ≥250 mg/dL, after which the animals were assigned to receive the respective doses of 6-gingerol [14].

At the conclusion of the experiment, rats were euthanized under deep anesthesia via intraperitoneal injection of ketamine/xylazine (87 mg/kg and 13 mg/kg, respectively). Blood was obtained through cardiac puncture into sterile tubes. Serum was separated by centrifugation at 2000 rpm for 10 minutes at 4°C and stored at -20°C pending further assays. Hearts were promptly excised, rinsed in ice-cold saline, weighed, and the heart weight-to-body weight (HW/BW) ratio was determined. Tissue samples were then preserved in Eppendorf tubes. A portion was frozen at -80°C for biochemical evaluations, while the remainder was immersed in 4% formaldehyde for histopathological examination.

Assessment of glycemia, blood pressure, and cardiac markers

Fasting serum glucose concentrations were determined using an Autocheck® Glucare glucometer (Medical Technology Promedr, St. Ingbert, Germany). Serum insulin levels were quantified via ELISA kits. Insulin resistance was estimated using the homeostasis model assessment (HOMA-IR) formula: [fasting glucose (mg/dL) × fasting insulin (μIU/mL)] / 405.

Blood pressure measurements involved the non-invasive tail-cuff technique, performed in triplicate. Rats were habituated in restrainers within a calm, heated setting for 10–15 minutes prior to recording. An occluding cuff equipped with a sensor was positioned at the tail base, inflated to block circulation, and slowly deflated while monitoring pulse resumption. Systolic (SBP) and diastolic (DBP) values were captured automatically, and the mean of three stable consecutive readings was calculated. Mean arterial pressure (MAP) was derived as DBP + 1/3 (SBP – DBP). Serum creatine kinase-MB (CK-MB) activity was assessed colorimetrically by measuring absorbance at 340 nm, while cardiac troponin-I (cTn-I) concentrations were determined using an ELISA kit [17].

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

Heart tissue specimens (100 mg) were homogenized (10% w/v) in normal saline using an Ultra Turrax® device, followed by centrifugation at 3000 rpm for 10 minutes at 4°C. The resulting supernatant was used for assays. Glutathione peroxidase (GPx) and tumor necrosis factor-α (TNF-α) concentrations were evaluated with ELISA kits. Malondialdehyde (MDA) content was quantified via a lipid peroxidation assay kit per the supplier's protocol. Absorbance readings were taken at 530 nm using a spectrophotometer, and values were derived from a standard calibration curve.

Quantification of IRS-1, PI3K, Akt, and GLUT-4 proteins in cardiac tissue

Protein expression of insulin receptor substrate-1 (IRS-1), phosphoinositide 3-kinase (PI3K), protein kinase B (Akt), and glucose transporter-4 (GLUT-4) in heart samples was measured using specific ELISA kits as per instructions. Briefly, 100 mg of tissue was rinsed in cold phosphate-buffered saline, homogenized in chilled manufacturer-supplied lysis buffer, and centrifuged at 10,000 × g for 10–15 minutes at 4°C. The cleared supernatant was promptly analyzed by ELISA.

Western blot analysis for AMPK, PGC-1α, and SIRT-1 expression in cardiac tissue

Heart tissue (100 mg) was minced and homogenized in ice-cold Tris-based buffer (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, and 0.01 mg/mL aprotinin). Homogenates were centrifuged at 3000 rpm for 10 minutes at 4°C, and supernatants were aliquoted and stored at -80°C. Total protein was quantified using the bicinchoninic acid (BCA) method. Equivalent protein loads (50 µg) were resolved by SDS-PAGE (Bio-Rad, USA) and blotted onto nitrocellulose membranes. Membranes were blocked with 5% non-fat dry milk (for PGC-1α and SIRT-1) or 5% bovine serum albumin (for AMPK and phospho-AMPK) in Tris-buffered saline with 0.1% Tween-20. Primary antibodies were applied overnight at 4°C (1:1000 dilution), followed by horseradish peroxidase-conjugated secondary antibody and chemiluminescent detection (Amersham Biosciences, UK). GAPDH served as the loading control. Band intensities were analyzed densitometrically using ImageJ software.

Histopathological examination

Cardiac hypertrophy was assessed morphologically in myocardial sections. Excised hearts were sectioned transversely into 5-µm slices, fixed in 10% formalin, and paraffin-embedded using routine procedures. Hematoxylin and eosin (H&E) staining was performed to visualize cardiomyocyte structure. Cross-sectional areas of individual cardiomyocytes were measured and quantified with ImageJ software.

Statistical analysis

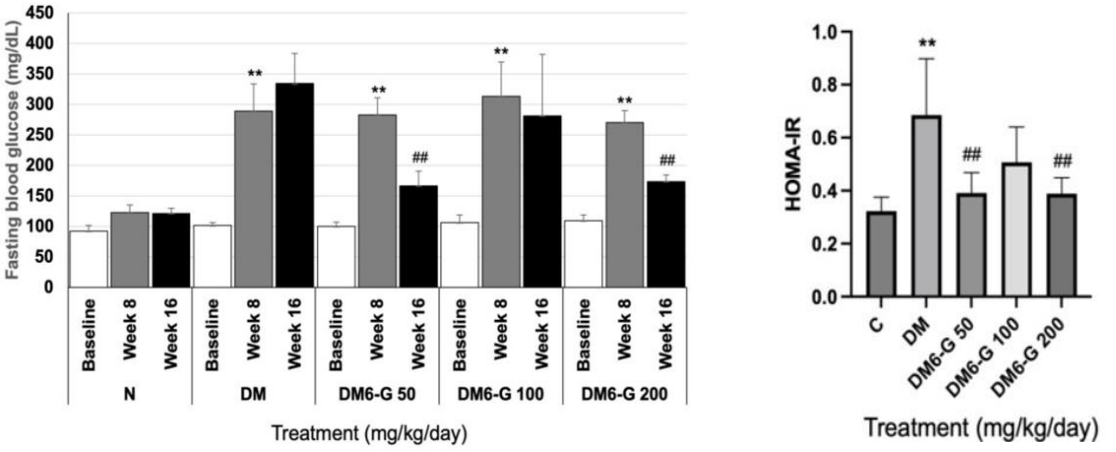
Results are expressed as mean ± standard error of the mean. Normality was verified using the Shapiro-Wilk test. Differences among groups were evaluated by one-way analysis of variance (ANOVA) with Tukey's post hoc test. Statistical computations were conducted in SPSS version 23.0, with significance set at $p < 0.05$.

Results and Discussion

The present research revealed that 6-gingerol, particularly across daily doses of 50 to 200 mg/kg, markedly reduced cardiomyocyte enlargement and inflammatory responses in rats with diabetes induced by prolonged exposure to a high-fat diet and elevated fructose intake over 16 weeks. Additionally, treatment with 6-gingerol for 8 weeks effectively lessened insulin resistance, as shown by lower HOMA-IR values and fasting glucose concentrations, along with diminished CK-MB and cTnI concentrations. It also suppressed oxidative damage in cardiac tissue, indicated by reduced MDA concentrations and higher GPx activity. This work further demonstrated increased expression of phosphorylated AMPK, SIRT-1, and PGC-1α proteins, accompanied by improved components of the insulin signaling cascade after 6-gingerol administration.

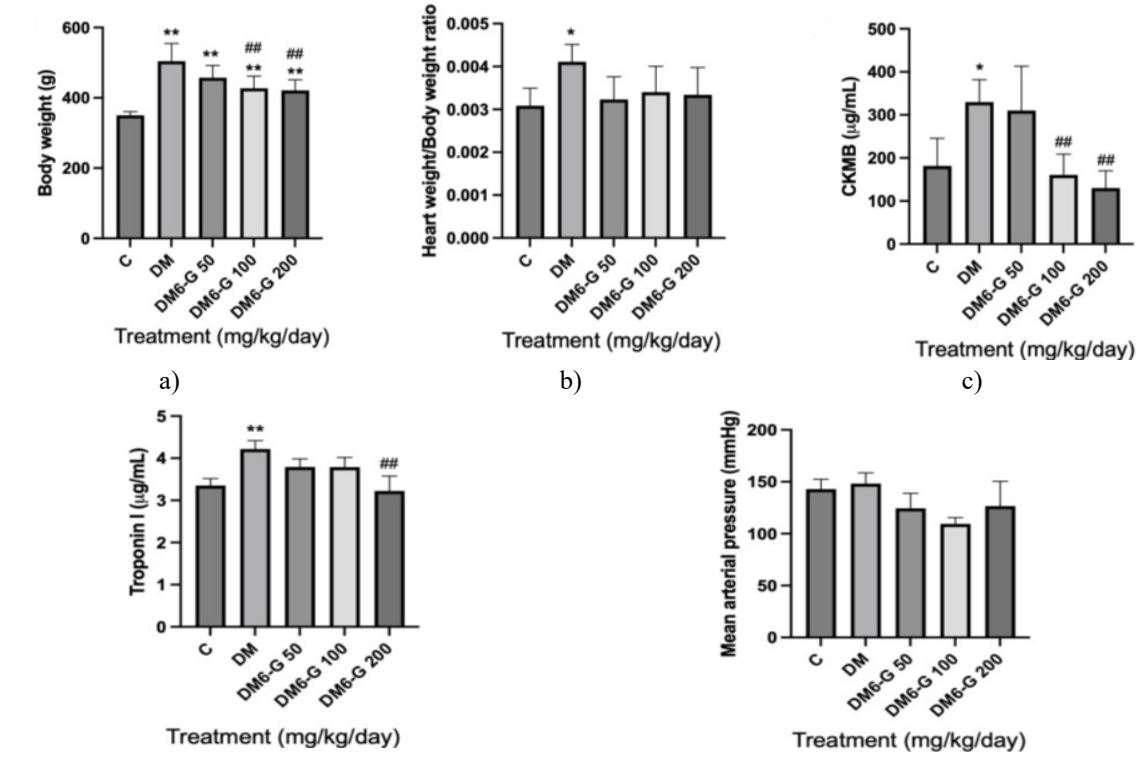
Prolonged intake of high-fat foods and excessive fructose is recognized to promote weight gain, resistance to insulin, persistent oxidative damage, and inflammation, all of which contribute to the development of cardiovascular complications in diabetes [5, 18]. Research has established that oxidative stress drives the onset and worsening of insulin resistance in type 2 diabetes by impairing key insulin transduction elements [5, 10, 11]. In this experiment, the 16-week regimen of high-fat feeding combined with fructose led to elevated fasting glucose **(Figure 1a),** heightened insulin resistance **(Figure 1b),** greater body mass **(Figure 2a),** and evidence of oxidative imbalance reflected in higher MDA **(Figure 3a),** lower GPx **(Figure 3b),** and raised TNF-α concentrations (**Figure 3c).** Notably, 6-gingerol administration reversed these abnormalities, with the most pronounced benefits observed at 200 mg/kg/day relative to the untreated diabetic group. Consistent with earlier findings, gingerol has been reported to lower circulating glucose, counteract oxidative stress, and improve insulin sensitivity [19, 20]. Moreover, the diabetic group exhibited an increased heart-to-body weight ratio compared to controls **(Figure 2b),** signifying cardiac hypertrophy, even without a notable increase in mean arterial pressure **(Figure 2e).** Treatment with 6-gingerol normalized these indices, though the changes did not reach statistical significance. Additional investigations are warranted to clarify if 6-gingerol can influence blood pressure regulation.

Excessive reactive oxygen species (ROS) generation is a defining feature of heart-related disorders. The AMP-activated protein kinase (AMPK), a primary regulator of cellular energy and metabolism, has emerged as a sensor of redox status that supports cardiac homeostasis and slows disease progression [21].



a) b)

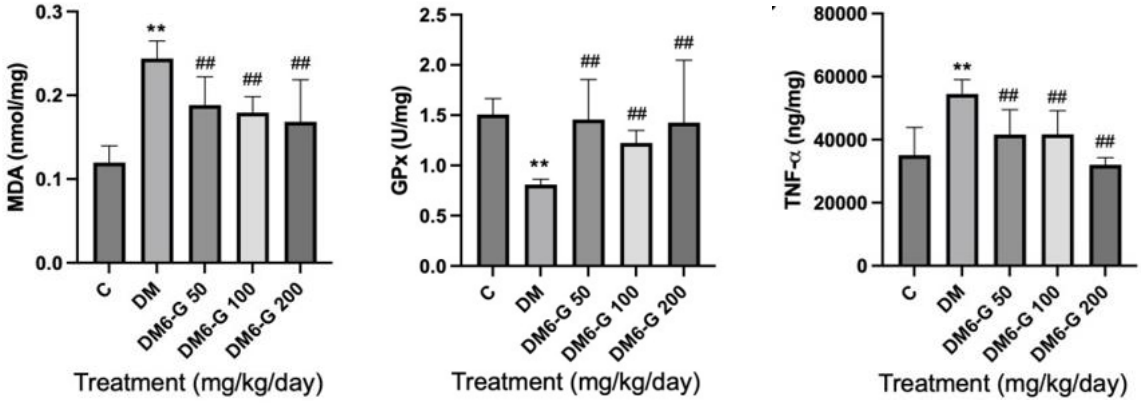
Figure 1. Reduction in fasting blood glucose concentrations (a) and homeostasis model assessment of insulin resistance (HOMA-IR) values (b). C: normal control group; DM: untreated diabetic group; DM6-G 50, DM6-G 100, and DM6-G 200: diabetic animals receiving 6-gingerol treatment at daily doses of 50, 100, and 200 mg/kg, respectively. Values are presented as mean ± standard error of the mean (SEM). **p < 0.01 versus control group; ##p < 0.01 versus untreated diabetic group.



a) b) c)

d) e)

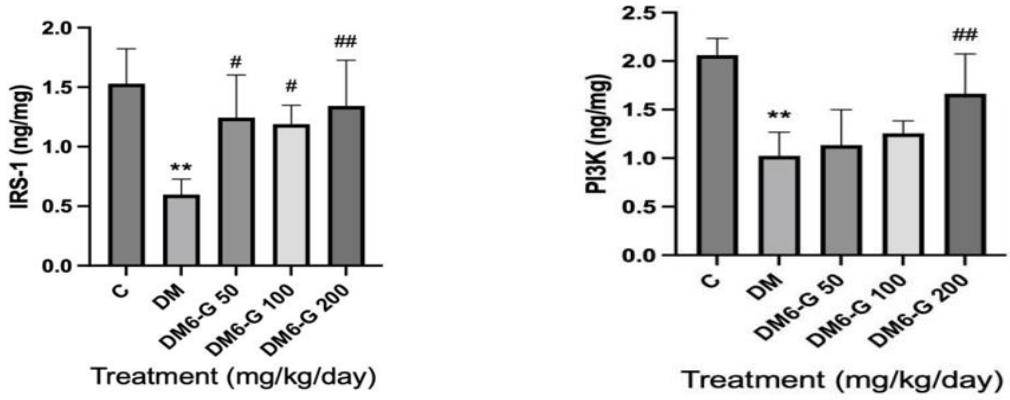
Figure 2. Influence of 6-gingerol administration on body weight (a), heart-to-body weight ratio (b), serum creatine kinase-MB (CK-MB) activity (c), cardiac troponin I levels (d), and mean arterial blood pressure (e) in the experimental groups. C: normal control; DM: untreated diabetic rats; DM6-G 50, DM6-G 100, and DM6-G 200: diabetic rats receiving daily oral doses of 6-gingerol at 50, 100, and 200 mg/kg, respectively. *p < 0.05 and **p < 0.01 versus control group; ##p < 0.01 versus untreated diabetic group.



a) b) c)

Figure 3. Influence of 6-gingerol administration on markers of oxidative stress and inflammation in cardiac tissue: malondialdehyde (MDA) content (a), glutathione peroxidase (GPx) activity (b), and tumor necrosis factor-α (TNF-α) levels (c) across the experimental groups. C: normal control group; DM: untreated diabetic group; DM6-G 50, DM6-G 100, and DM6-G 200: diabetic animals receiving daily 6-gingerol at 50, 100, and 200 mg/kg, respectively. Results are shown as mean ± standard error of the mean (SEM). **p < 0.01 versus control group; ##p < 0.01 versus untreated diabetic group.

Additionally, AMPK and SIRT-1 act as energy-sensing proteins that overlap in downstream targets and mutually modulate one another to suppress reactive oxygen species (ROS) generation and pro-inflammatory cytokines [6, 16, 21, 22]. SIRT1 plays a key role in controlling numerous essential physiological processes, including oxidative stress, cell death, and autophagy, whereas PGC-1α is highly expressed in cardiomyocytes and contributes significantly to cardiac protection by promoting the transcription of antioxidant genes [23-25]. Research by Tian *et al*. indicated that AMPK can affect the expression of PGC-1α and SIRT1 downstream, while SIRT1 may conversely participate in stimulating AMPK and PGC-1α [26]. Recent studies have demonstrated that a diet rich in fat and fructose elevates oxidative stress and reduces the expression of AMPK, PGC-1α, and SIRT-1 [27]. Evidence suggests that AMPK activation can be modulated by various hormones and phytochemicals. Certain plant-derived compounds have been reported to restore fructose-induced reductions in phosphorylated AMPK in cardiac cells and diabetic models [28, 29]. Furthermore, ginger and its active component 6-gingerol have been found to enhance AMPK phosphorylation as well as PGC-1α and SIRT1 expression, thereby protecting against arsenic trioxide-triggered cardiotoxicity and mitigating obesity in animal models [30, 31]. In agreement with these findings, our results showed that eight weeks of 6-gingerol treatment at all tested doses markedly reduced oxidative stress and inflammation while elevating protein expression of phosphorylated AMPK (p-AMPK), PGC-1α, and SIRT-1 in rats maintained on a high-fat, high-fructose diet **(Figures 5a-5d).**



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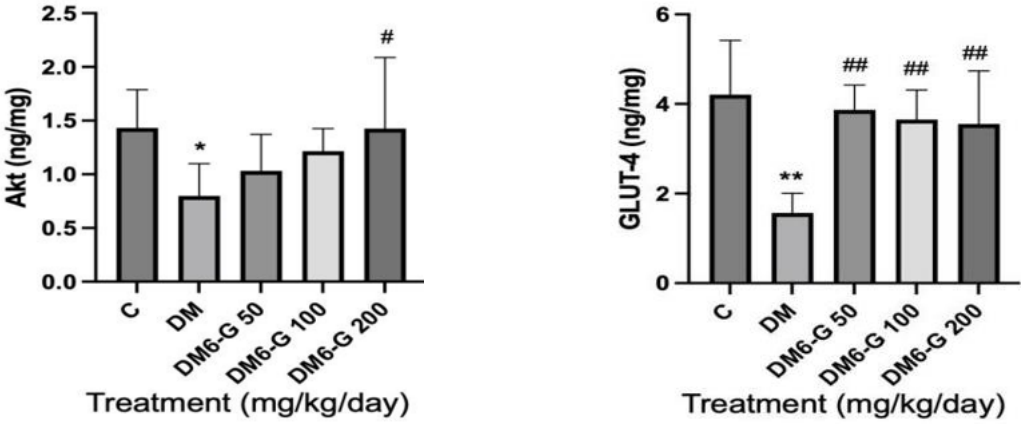
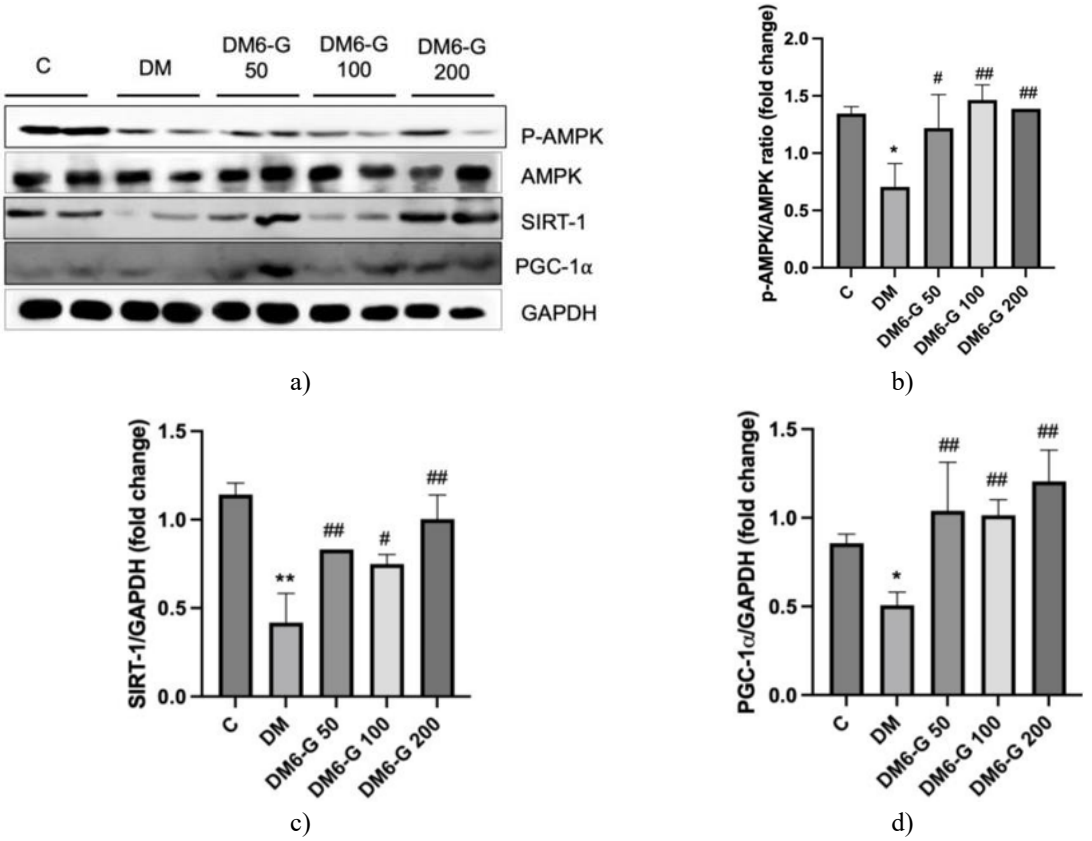


c) d)

Figure 4. Impact of 6-gingerol treatment on protein expression levels in cardiac tissue: IRS-1 (a), PI3K (b), Akt (c), and GLUT-4 (d). C: normal control group; DM: untreated diabetic group; DM6-G 50, DM6-G 100, and DM6-G 200: diabetic animals treated with daily doses of 6-gingerol at 50, 100, and 200 mg/kg, respectively. Data are presented as mean ± standard error of the mean (SEM). *p < 0.05 and **p < 0.01 versus control group; #p < 0.05 and ##p < 0.01 versus untreated diabetic group.



a) b)

c) d)

Figure 5. Influence of 6-gingerol on the AMPK/SIRT-1/PGC-1α pathway in cardiac tissue; representative Western blot images showing protein levels of phosphorylated AMPK (p-AMPK), SIRT-1, and PGC-1α (a); quantification demonstrating that 6-gingerol enhanced AMPK phosphorylation (b) as well as protein expression of SIRT-1 (c) and PGC-1α (d). C: normal control group; DM: untreated diabetic group; DM6-G 50, DM6-G 100, and DM6-G 200: diabetic animals receiving daily 6-gingerol treatment at 50, 100, and 200 mg/kg, respectively. Results are presented as mean ± standard error of the mean (SEM). *p < 0.05 and **p < 0.01 versus control group; #p < 0.05 and ##p < 0.01 versus untreated diabetic group.

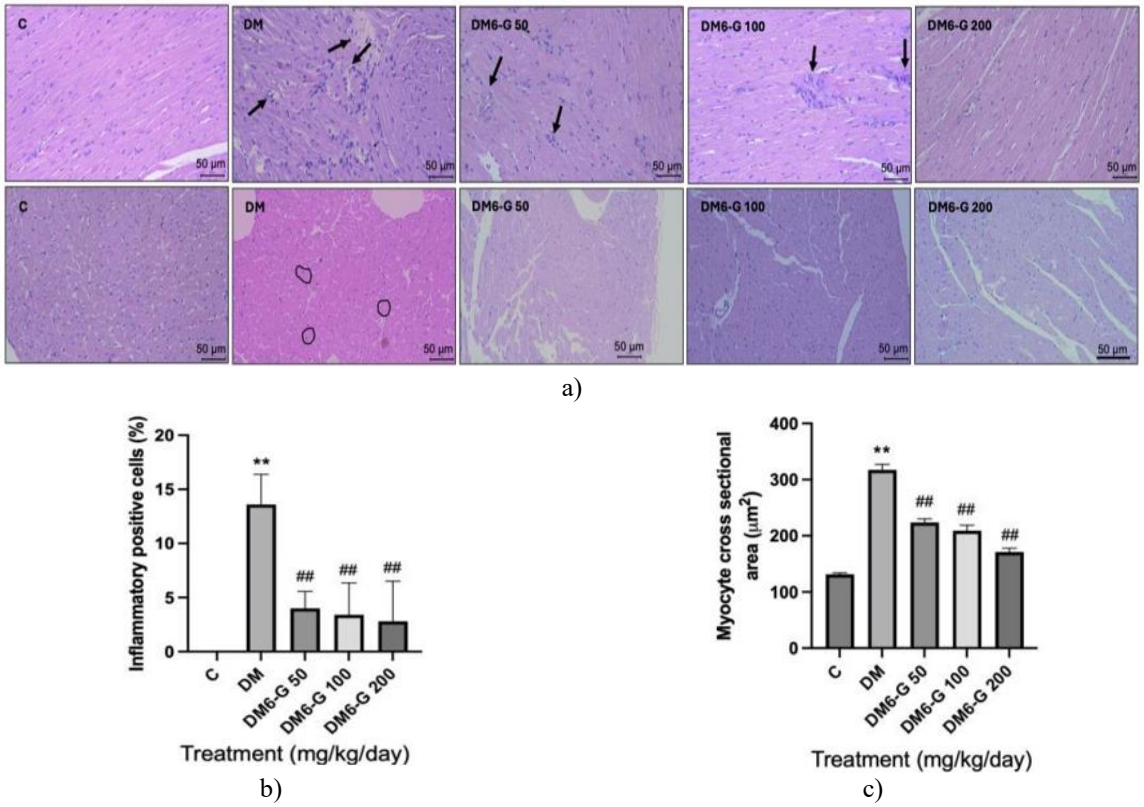


Figure 6. Attenuation of cardiac inflammation and cardiomyocyte hypertrophy by 6-gingerol in diabetic rats; representative hematoxylin and eosin (H&E)-stained myocardial sections (magnification ×400), with black arrows indicating infiltrating inflammatory cells (a); quantification of inflammatory cell infiltration (b) and cardiomyocyte cross-sectional area (outlined in black) (c); scale bar represents 50 μm. C: normal control group; DM: untreated diabetic group; DM6-G 50, DM6-G 100, and DM6-G 200: diabetic animals administered 6-gingerol orally at daily doses of 50, 100, and 200 mg/kg, respectively. Values are presented as mean ± standard error of the mean (SEM). **$p < 0.01$ versus control group; ##$p < 0.01$ versus untreated diabetic group; n=6.

Furthermore, our findings indicate that oxidative stress induced by high-fat diet (HFD) and fructose intake impairs insulin signaling, leading to the development of insulin resistance. A key pathway associated with insulin resistance is PI3K/Akt, which plays a critical role in various cellular functions, particularly glucose transport [15]. PI3K activation is crucial for facilitating glucose uptake, with Akt acting as the primary downstream effector [15]. In cardiomyocytes, GLUT4 serves as the main glucose transporter, regulating cardiac energy metabolism and cellular growth [32]. Additionally, elevated PGC-1α expression has been demonstrated to promote GLUT4 mRNA transcription via coactivation of MEF2C in L6 myotubes [33].

In the present investigation, protein levels of IRS-1, PI3K, Akt, and GLUT4 in cardiac tissue were markedly reduced in rats with HFD- and fructose-induced insulin resistance compared to normal controls. Administration of 6-gingerol led to substantial increases in IRS-1 and GLUT4 protein expression at all tested doses **(Figures 4a and 4d).** The highest dose (200 mg/kg/day) notably elevated PI3K and Akt protein levels **(Figures 4b and 4c).** Comparable changes in these proteins have been observed in skeletal muscle of ob/ob mice [34]. In our earlier work, we demonstrated that 6-gingerol at 200 mg/kg/day enhanced pancreatic α- and β-cell populations, contributing to better glycemic regulation [35]. Thus, we propose that insulin resistance triggered by HFD and fructose disrupts glucose homeostasis and promotes oxidative stress, likely by suppressing PI3K/Akt and AMPK pathways, thereby hindering GLUT4 translocation to the plasma membrane for glucose uptake. These biochemical observations were corroborated by histological examinations, which revealed that 16 weeks of HFD and fructose feeding compromised cardiac architecture, manifesting as enlarged cardiomyocytes and heightened inflammatory infiltration in the DM group. Treatment with 6-gingerol across all doses effectively ameliorated these pathological changes **(Figures 6a-6c).**

Conclusion

Treatment with 6-gingerol (50, 100, and 200 mg/kg/day) enhances GLUT4 expression through upregulation of PI3K/Akt and AMPK/SIRT-1/PGC-1α signaling proteins, thereby lowering HOMA-IR scores, suppressing inflammatory responses, and boosting cardiac antioxidant enzyme activity in rats with diabetes induced by high-fat and high-fructose intake. These molecular improvements translate to preserved myocardial structure and decreased inflammatory cell presence. Additional research is warranted to clarify the pharmacokinetics of 6-gingerol and optimal treatment durations. A key limitation of this work is the absence of direct assessments of cardiac performance, such as echocardiographic or hemodynamic evaluations. Nonetheless, the marked declines in cTnI and CK-MB in treated animals offer indirect support for attenuated myocardial damage, suggesting a cardioprotective effect of 6-gingerol in hypertrophic cardiomyopathy models. Upcoming studies with comprehensive in vivo functional testing will be vital to confirm its clinical promise in diabetic cardiomyopathy.

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Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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