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Dapagliflozin Protection against Myocardial Ischemia by Modulating Sodium-glucose Transporter 2 Inhibitor, Silent Information Regulator 1, and Fatty Acid Synthase Expressions

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Abstract

BACKGROUND: The emerging role of sodium-glucose transporter 2 (SGLT2) inhibitors drugs as potential therapeutic agents in myocardial ischemic (MI) injury treatment has raised the concern for possible mechanisms of action.

AIM: The current experimental study aimed to investigate the possible protective effects of dapagliflozin (DAPA) a SGLT2i, on isoproterenol (ISO)-induced MI in rats.

MATERIALS AND METHODS: Thirty Wistar rats were divided randomly and equally into three groups. Group 1 (control group): Received 1.0 mL of normal saline through an orogastric tube for 14 days. Group 2 (ISO group): Received 1.0 mL of normal saline orally through an orogastric tube for 14 days. In the last 2 days (days 13 and 14), ISO (100 mg/kg) was freshly dissolved in normal saline and injected subcutaneously once daily. Group 3 (ISO + DAPA-treated group): Received DAPA 1.0 mg/kg/day orally for 14 days. In the last 2 days (days 13 and 14), ISO (100 mg/kg) was introduced like that described in Group 2.

RESULTS: DAPA protects MI development by reversal of blood pressure changes, electrocardiographic alterations, stabilization of cardiac enzymes, inflammation restoration, oxidative stress, and lipid profile. SGLT2 was overexpressed in the ISO-induced MI, which declined in the ISO + DAPA group. Moreover, DAPA induced silent information regulator 1 (SIRT1)/fatty acid synthase (FASN) overexpression in ISO-induced MI. DAPA could have a potential protective role against acute MI.

CONCLUSION: DAPA protects against acute MI by modulating SIRT1 and FASN expression in cardiac muscles, suppressing oxidative stress, and downregulating inflammatory mediators.

Introduction

Acute myocardial ischemia (MI) is a critical cardiovascular disorder estimated as the global cause of morbidity and mortality [1]. MI results from coronary artery obstruction because of atherosclerotic clots or artery spasms [2]. Obstruction of the coronary arteries results in MI due to oxygen and nutrient deprivation [3]. Furthermore, the release of reactive oxygen species (ROS) depletes the antioxidant enzymes inducing lipid peroxidation and protein and nucleic acid degeneration, which worsens the cardiac inflammatory reaction and cardiomyocyte apoptosis [4]. The activation of the nuclear factor-kappa beta (NF-κB) pathway has been recognized as a key factor in cardiac remodeling failure, inflammation, and fibrosis through the initiation of innate immunity and the inflammatory process [5]. In addition, the lack of regenerative capacity of the heart cells interferes with the regenerative process with the development of myocardial fibrosis, the major pathogenesis of ischemic cardiomyopathy, and systolic and diastolic dysfunctions [6].

The emerging role of sodium-glucose transporter 2 (SGLT2) inhibitors as potential therapeutic agents in MI treatment has raised concerns for possible mechanisms of action [7]. Moreover, SGLT2 is a transmembrane protein that is pivotal in renal sodium and glucose reabsorption [8]. In addition, SGLT2 inhibitors are used as monotherapy or combination therapy with other antidiabetic drugs to control hyperglycemia [9]. Along with the hypoglycemic effect of the SGLT2 inhibitors, several studies have correlated SGLT2 inhibitors to have antihypertensive effects through systolic and diastolic blood pressure reduction without altering the heart rate or fainting episodes [10]. The SGLT2 expression in the heart tissue is a matter of controversy. Thus, SGLT2 inhibitors could exert their function indirectly through autophagy downregulation [11].

Silent information regulator 1 (SIRT1) is a histone deacetylase of nicotinamide adenine dinucleotide (NAD^+) found inside the nucleus and has a crucial role in cell proliferation, differentiation, and autophagy [12]. SIRT1 promotes the activity

of antioxidant enzymes and interacts directly with NF-κB, inhibiting pro-inflammatory signaling in heart tissue [5]. In addition, SIRT1 activation maintains the proper functioning of the mitochondria and peroxisomes and reprograms the metabolic pathways mainly in gluconeogenesis and fatty acid oxidation. The two common downstream effectors of SIRT1 stimulation are the peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1α) and fibroblast growth factor 21 (FGF21). Fatty acid synthase enzyme (FASN) is a member of lipogenic pathways that are downregulated by FGF21 activation [13]. In addition, FASN is a housekeeping protein that can be used for energy storage, membrane assembly, and repair and secretion in the form of lipoprotein triglycerides during fasting [14]. However, prolonged FASN activation could result in inflammation, lipid peroxidation, and fibrosis [15]. However, the role of FASN activation in cardiac tissue is a matter of controversy [16].

The emerging role of SGLT2is drugs as potential therapeutic agents in MI injury treatment despite the absence of known heart receptors has raised the concern for possible mechanisms of action. Therefore, the current experimental study aimed to evaluate the protective effects of dapagliflozin (DAPA) a SGLT2 inhibitor in a rat model of MI induced by isoproterenol (ISO). In addition, the immunohistochemical expression of SIRT1 and FASN was assessed as a possible mechanism of DAPA-induced protective function.

Materials and Methods

Animals

This study followed the institutional experimental animal care and the ethical committee [IRB No: 00269/2021]. The Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines were rigorously adhered during the execution of all operations described in this publication [17]. Rats were kept dry, insulated with paper towels, and warmed with a heated towel (maintained at 37°C) throughout the trial to avoid heat loss.

The sample size was calculated at a 95% confidence interval and study power of 80% (0.8). Moreover, the sample size was 30 male Wistar albino rats with the same age (weight, 170–200 g). Rats were kept randomly inside animal cages at 23°C for a 12 h light/dark cycle with easy access to normal laboratory food and water.

Drugs

ISO hydrochloride powder (Sigma-Aldrich, St. Louis, MO, USA), DAPA (FORXIGA®, AstraZeneca Pharmaceuticals LP, Bangkok, Thailand), and phosphate buffer saline (PBS) (Biodiagnostic CO, Dokki, Giza, Egypt) were used.

Study design and MI induction

Rats were randomized into three experimental groups (n = 10/group) as follows:

Group 1 (control group): Received 1.0 mL of normal saline through an orogastric tube for 14 days.

Group 2 (ISO group): Received 1.0 mL of normal saline orally through an orogastric tube for 14 days. In the last 2 days (days 13 and 14), ISO (100 mg/kg) was freshly dissolved in normal saline and injected subcutaneously once daily [18].

Group 3 (ISO + DAPA-treated group): Received DAPA 1.0 mg/kg/day orally for 14 days. In the last 2 days (days 13 and 14), ISO (100 mg/kg) was introduced like that described in Group 2. On day 15, the following measurements were recorded:

The systolic blood pressure (SBP) of all groups was measured by tail-cuff plethysmography (Harvard Apparatus Ltd., Edenbridge, England) in conscious rats. To obtain consistent results, the animals must be handled, warmed, and restrained. Before measurement, the rats were trained to restrain themselves for 30 min/day for 2 days.

After 5 min of acclimation, we performed five consecutive measurements with a 1 min interval between returns. To obtain a consistent reading, the average of the five readings was taken [19].

Electrocardiography (ECG) monitoring was performed following the protocol of Balea *et al*. [20]. The animals were administered xylazine (2.6 mg/kg, i.p.) and ketamine (26 mg/kg, i.p.) as general anesthesia. The animals were then placed supine on a board for 15 min. Each rat's paw pads were fitted with electrodes, and an ECG was recorded in lead II using the BIOPAC MP36 system (Goleta, CA, USA). Moreover, ECG was analyzed using BIOPAC Student Lab 3.7.7 (Goleta, CA, USA). RR intervals (in millisecond), PR segments (in millisecond), QRS duration (in millisecond), QT intervals (in millisecond), and ST-segment alterations (in millivolts) were calculated. The RR interval was used to compute the heart rate (HR; in beats per minute) following the formula: HR = 60,000/RR [21].

Blood sampling and tissue homogenate

All rats were sacrificed by head decapitation, and the venous blood samples of each rat were collected using heparinized capillary tubes from the retro-orbital plexus. The collected samples were centrifuged at 500×*g* for 20 min at 4°C to obtain serum and kept at −80°C. The heart of each rat was

then dissected out, washed with saline, socked, and weighed immediately. A small part of heart tissue was stored in the formaldehyde (40%) for histopathology. Part of the cardiac tissue was used to prepare tissue homogenate. The homogenate was prepared with 0.1 ± 0.05 g of myocardial tissue ground in 200 μL of PBS. The final tissue homogenate amount in each sample was adjusted to 10% by adding 700 μL of PBS. The homogenate was then centrifuged at 800×*g* for 10 min then stored at −80°C for biochemical analysis.

Determination of serum cardiac biomarkers

Creatine kinase-MB (CK-MB) and cardiac troponin I (cTnI) were measured by the enzyme-linked immunoassay (ELISA) technique (Chongqing Biospes Co., Ltd., Chongqing, China; CK-MB BEK1248, cTnI BEK1253), following the manufacturer's procedure.

Glucose and lipid levels were measured by an enzymatic method

A hand-held glucometer was used to measure blood glucose levels (OneTouch Verio IQ Lifescan, Johnson & Johnson Company). Spectrophotometric measurements of plasma cholesterol and triglyceride (TG) concentrations were performed using commercial kits (DiaSys Diagnostic Systems GmbH, Holzheim, Germany; cholesterol FS 10130021 and TG FS 10571 021).

Determination of oxidative stress markers and antioxidant levels

Malondialdehyde (MDA), superoxide dismutase (SOD), and reduced glutathione (GSH) enzymes were measured in tissue homogenates by colorimetric assay using available commercial kits (Bio Diagnostic Co., Dokki, Giza, Egypt; MDA MD 25 29, SOD SD 25 21, GSH GR 25 11). The results were expressed in units in milligram protein.

Determination of cardiac inflammatory markers

Tumor necrosis factor-α (TNF-α) was measured using rat ELISA kits (ERT2010-1, Assaypro LLC, Saint Charles, MO, USA), and interleukin-6 (IL-6) was measured using rat ELISA kits (ab100772, Abcam, Cambridge, UK). The results were expressed as picograms per milliliter.

Pathological assessment

Heart tissue was collected from sacrificed rats. Representative sections were processed and embedded in paraffin blocks after fixation in neutral buffered formalin for 24 h.

Hematoxylin and eosin (H&E)

Sections of 4–5 μm thickness were cut, deparaffinized, dehydrated, and stained with H&E for the assessment of the histopathological changes.

Masson trichrome (MTC) stain

MTC staining was performed to assess collagen deposition in the heart muscles following a standard protocol [22]. The collagen fibers were stained blue against red-stained muscle fibers.

Immunohistochemistry technique

A rabbit polyclonal SGLT2 antibody (A20271) diluted at 1:100 was obtained from ABclonal, Woburn, Massachusetts, USA. SIRT1 antibody diluted at 1:50 (YPA2140) was obtained from Chongqing Biospes Co., Ltd., Chongqing, China, and FASN antibody (sc-55580) diluted at 1:200 was obtained from Santa Cruz Biotechnology Inc., Dallas, Texas, USA. After tissue deparaffinization and rehydration, antigen retrieval using a high pH EDTA solution (Dako, Ref K8000, Glostrup, Denmark) was performed, followed by cooling at room temperature. The primary antibodies were incubated overnight at 4°C. Secondary antibody using UltraVision Detection System: Anti-Polyvalent HRP/DAB, ready-to-use, NeoMarker was applied, and staining was visualized using a DAB chromogen substrate and Mayer's hematoxylin as a counterstain. Positive and negative controls were used in each run.

Assessment of primary antibodies

The SGLT2 and FASN expressions showed cytoplasmic/membranous localization, and SIRT1 showed both nuclear and cytoplasmic localization [23], [24], [25]. Histoscore system was calculated as follows: Strong intensity (3) × percentage + moderate intensity (2) × percentage + mild intensity (1) × percentage + negative staining (0) × percentage. A final score ranged from 0 to 300 [23].

Statistical analysis

The collected data of the BP, ECG, CK-MB, cTnI, MDA, SOD, GSH, TNF-α, and IL-6 levels were recorded and tabulated. Moreover, histopathological and immunohistochemical data were recorded. All findings were analyzed using one-way analysis of variance (ANOVA) and the Bonferroni *post hoc* test and expressed as mean ± standard error of mean

(SEM), while categorical data were analyzed using Monte Carlo test. GraphPad Prism 9 was used to statistically analyze the data (GraphPad Software, San Diego, CA, USA). Statistical significance was defined as a value of $p < 0.05$.

Results

Blood pressure

The SBP of the ISO group was significantly decreased compared with those of the control group (87 ± 0.68 vs. 147.4 ± 0.91 mmHg; $p < 0.05$). Moreover, the ISO + DAPA-treated group showed a significant increase in the SBP (104.5 ± 0.92 vs. 87 ± 0.68 mmHg) compared with the ISO group ($p < 0.05$, Figure 1).

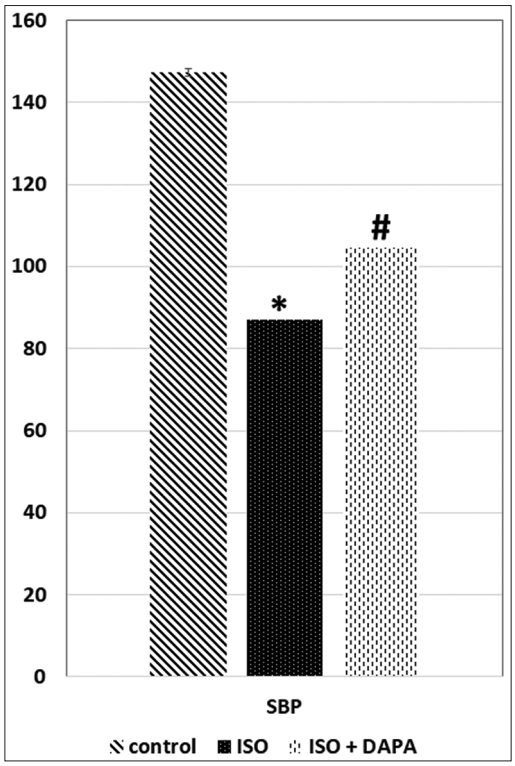

*Figure 1: Dapagliflozin elevates ISO induced reduction of systolic blood pressures. Data were expressed as mean ± SEM (n = 10 for each group). BP, blood pressure; SPB, systolic blood pressure; ISO, isoproterenol; DAPA, dapagliflozin. *P < 0.05 vs. control; #P < 0.05 vs. ISO*

Electrocardiography

ECG tracing revealed normal heart activity in both the control and DAPA-treated groups. Significant ECG alterations with shortened RR interval and hence tachycardia, ST-segment elevation, QRS, and QT interval prolongation were observed in the ISO group compared with the control group ($p < 0.001$). However, such ECG abnormalities improved in the ISO + DAPA-treated group ($p < 0.001$) as demonstrated by the normalization of the RR interval, HR, ST-segment, and QT intervals when compared with the ISO group (Table 1).

Table 1: The electrocardiographic effect of dapagliflozin in isoproterenol-induced myocardial ischemic

Parameters	Group			p
	Control	ISO	ISO+DAPA	
HR (b/min)	382 ± 1.15	562.67 ± 1.76*	392 ± 0.58#	p1 < 0.001 p2 = 0.001 p3 < 0.001
RR (ms)	154.67 ± 0.88	104.67 ± 0.88*	154.00 ± 0.58#	p1 < 0.001 p2 = 0.614 p3 < 0.001
QRS (ms)	27.33 ± 0.88	32.00 ± 0.58*	28.00 ± 0.58#	p1 = 0.004 p2 = 0.587 p3 < 0.05
QT (ms)	46.00 ± 0.58	54.00 ± 0.58*	46.00 ± 1.53#	p1 = 0.001 p2 = 1.00 p3 = 0.001
ST (mV)	0.02 ± 0.00	0.04 ± 0.00*	0.03 ± 0.00#	p1 < 0.001 p2 = 0.007 p3 = 0.002

*Significantly different from control group, #significantly different from ISO group. HR, RR, QRS, QT, and ST intervals. Data were expressed as mean ± SEM and analyzed using one-way ANOVA followed by Bonferroni's *post hoc* test at $p < 0.05$. *n* = 10. p1 control regarding ISO. p2 control regarding ISO + DAPA. p3 ISO regarding ISO + DAPA. HR: Heart rate, b/min: Beat per minute, ms: Millisecond, mV: Millivolt, ANOVA: Analysis of variance, DAPA: Dapagliflozin, ISO: Isoproterenol, SEM: Standard error of mean.

Biochemical results

Regarding cardiac enzymes, the serum CK-MB (400.2 ± 39.72 vs. 153.9 ± 2.64 U/L) and cTnI (501.4 ± 2.37 vs. 57.7 ± 0.6 pg/mL) of the ISO group were significantly increased compared with those of the control group ($p < 0.05$ for both). However, the ISO + DAPA-treated group showed a significant decrease in the CK-MB (307 ± 3.51 vs. 400.2 ± 39.72 U/L) and cTnI (307 ± 3.51 vs. 501.4 ± 2.37 pg/mL) compared with the ISO group ($p < 0.05$ for both; Figure 2a and b).

Regarding serum lipid profile, both cholesterol (233.12 ± 1.10 vs. 145.5 ± 0.41 mg/dL) and TG (179.13 ± 1.71 vs. 120.56 ± 0.96 mg/dL) were significantly increased in the ISO group compared with the control group ($p < 0.05$ for both). Moreover, the ISO + DAPA-treated group showed a significant decrease in the cholesterol (191.39 ± 1.09 vs. 233.12 ± 1.10 mg/dL) and TG levels (119.12 ± 1.16 vs. 179.13 ± 1.71 mg/dL) compared with the ISO group ($p < 0.05$ for both; Figure 2c and d).

The MDA level in the ISO group was significantly increased (150.50 ± 1.32 vs. 85.6 ± 1.06 nmol/mg protein) compared with that in the control group ($p < 0.05$). The ISO + DAPA-treated group showed a significant decrease in the MDA (118.70 ± 1.29 vs. 501.4 ± 2.37 nmol/mg protein) compared with the ISO group ($p < 0.05$; Figure 3a). The SOD level (59.8 ± 1.03 vs. 79.8 ± 0.88 U/mg tissue protein) and GSH (2.75 ± 0.07 vs. 6.82 ± 0.15 nM/mg tissue protein) were significantly decreased in the ISO group compared with the control group ($p < 0.05$ for both). The ISO + DAPA-treated group showed a significant increase in the SOD (69.9 ± 1.24 vs. 59.8 ± 1.03 U/mg tissue protein) and GSH (3.87 ± 0.07 vs.

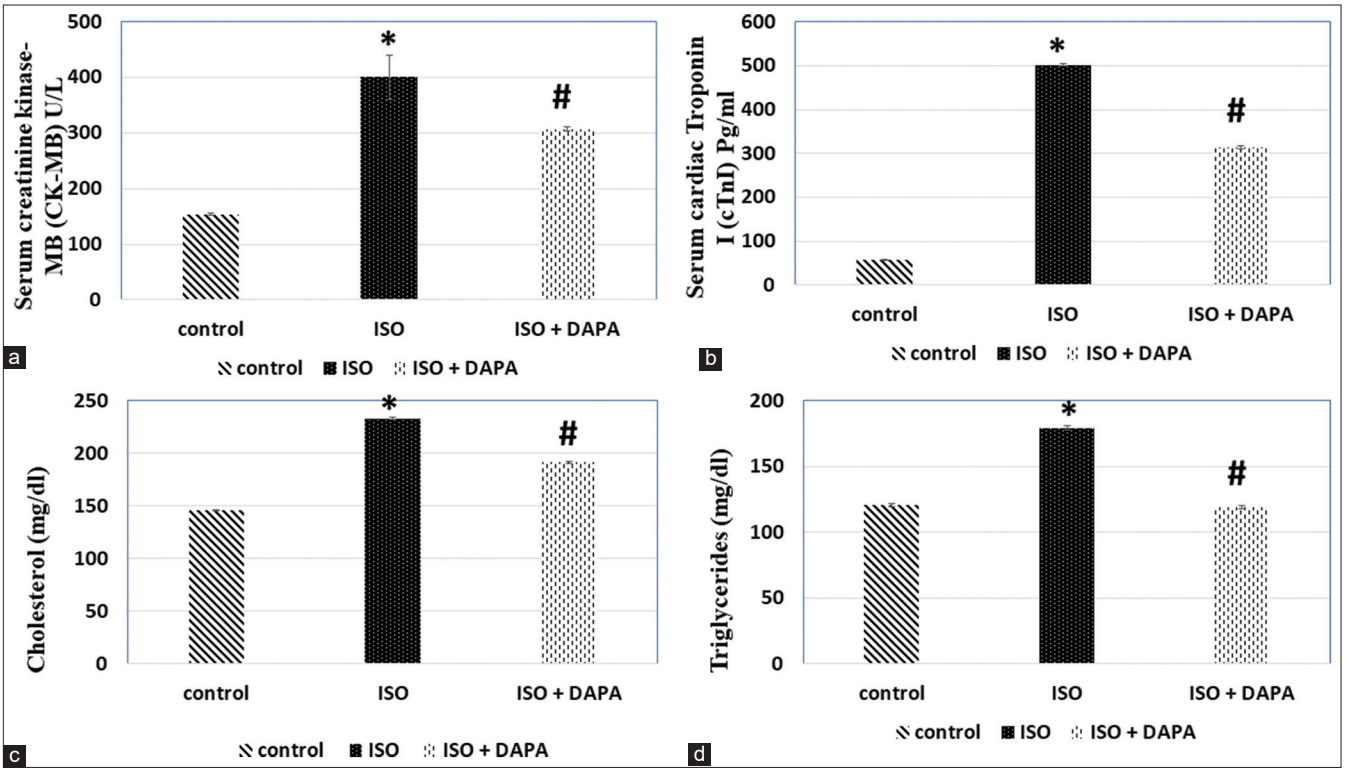

*Figure 2: Dapagliflozin reduces ISO-induced elevation of (a) Serum creatinine kinase-MB (CK-MB) U/L and (b) Serum cardiac Troponin I (cTnI) Pg/ml. Serum lipid levels. Dapagliflozin reduces ISO-induced elevation of cholesterol and triglycerides. (c) Cholesterol. (d) Triglycerides. Data were expressed as mean ± SEM (n = 10 for each group). ISO, isoproterenol; DAPA, dapagliflozin. *P < 0.05 vs. control; #P < 0.05 vs. ISO*

2.75 ± 0.07 nM/mg tissue protein) compared with the ISO group ($p < 0.05$; Figure 3b and c).

The inflammatory mediators' level was measured, and a significant TNF-α (140.11 ± 0.72 vs.

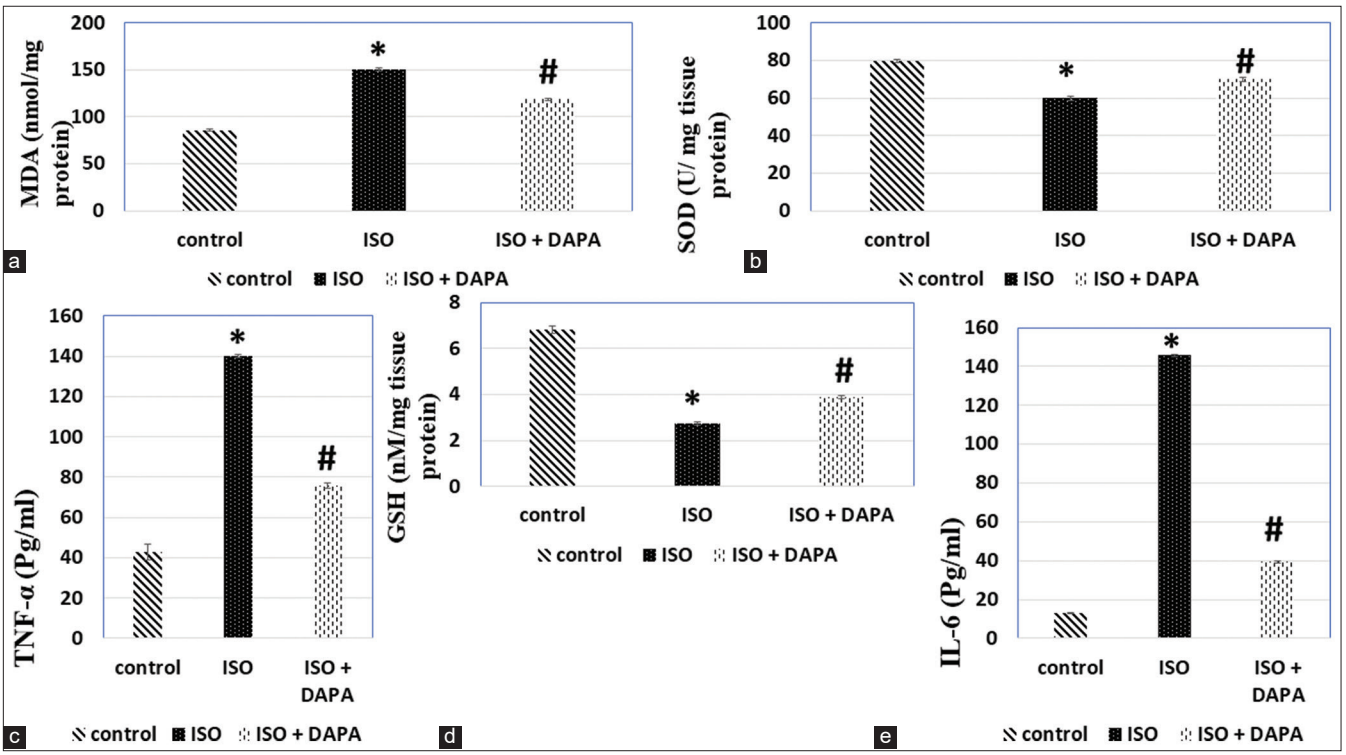

*Figure 3: Oxidative stress markers and antioxidant levels and cardiac inflammatory markers. Dapagliflozin reduces ISO-induced elevation of oxidative stress markers and prevents ISO-induced reduction of endogenous antioxidants in rat heart tissues. Also, Dapagliflozin reduces ISO-induced elevation of inflammatory markers in rat heart tissues. (a) Malondialdehyde (MDA). (b) Superoxide dismutase (SOD). (c) Reduced glutathione (GSH) (d) Tumor necrosis factor-α (TNF-α). e Interleukin-6 (IL-6). Data were expressed as mean ± SEM. (n = 10 for each group). ISO, isoproterenol; DAPA, dapagliflozin. *P < 0.05 vs. control; #P < 0.05 vs. ISO*

43.03 ± 3.87 pg/mL) and IL-6 (145.65 ± 0.65 vs. 12.88 ± 0.26 pg/mL) increase in the ISO group compared with the control group ($p < 0.05$ for both) were found. Moreover, the ISO + DAPA-treated group showed a significant decrease in TNF-α (75.74 ± 1.38 vs. 140.11 ± 0.72 U/mg tissue protein) and IL-6 (39.32 ± 0.62 vs. 145.65 ± 0.65 pg/mL) compared with the ISO group ($p < 0.05$ for both; Figure 3d and e).

Histopathological findings

Histological myocardium examination of both the control and DAPA-treated groups revealed a normal myofibrillar structure with clear transverse striations, centrally located nuclei, acidophilic sarcoplasm, and inflammatory infiltrate absence (Figure 4a and b). The ISO group showed marked disturbed and fragmented myocardial fibers with vacuolation, edema, peripheral pyknotic nuclei, and interstitial edema compared with the control group ($p < 0.001$). In addition, significant moderate congestion and inflammation in between myocardial cells were observed ($p < 0.001$ for both; Figure 4d and e). Moreover, the ISO + DAPA group revealed a significant improvement of the histopathological changes with mild myocardial damage and edema ($p = 0.003$ and $p = 0.012$) and complete inflammatory infiltrate clearing ($p < 0.001$), Figures 4g and h. Table 2 illustrated the histopathological changes in the studied groups.

MTC stain

No collagen fibers were noted between cardiac myocytes in both the control and DAPA-treated groups. Collagen deposition was highlighted by MTC in between myocardial fibers and the wall of blood vessels in the ISO group. However, a minimal degree of collagen deposition was observed in the ISO + DAPA-treated group (Figure 4c, f, and i).

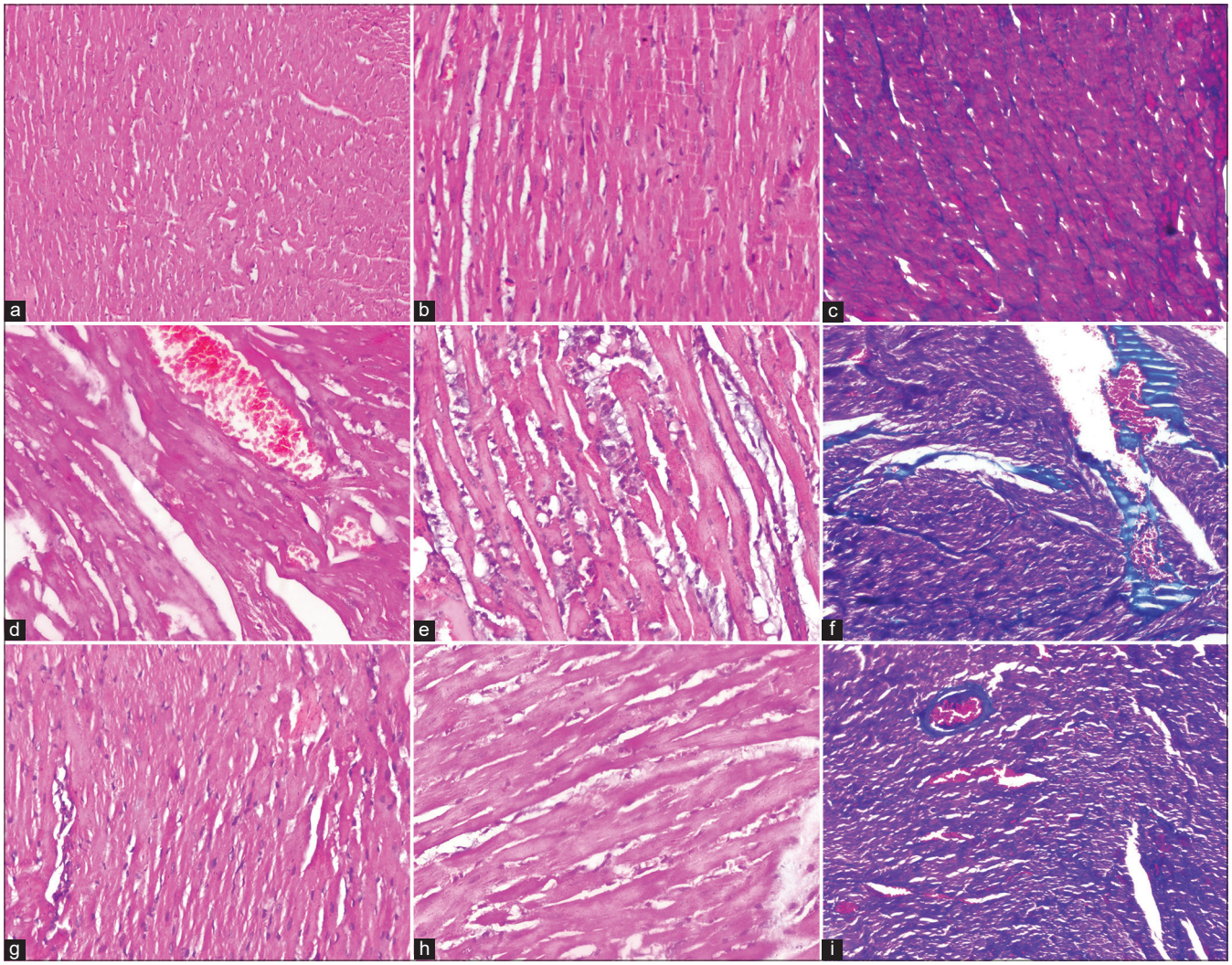

Figure 4: Histopathological changes of cardiac muscle. (a) The control group showed a normal myofibrillar structure with clear transverse striations (H&E, 100×). (b) The cardiac muscle showed the absence of inflammatory infiltrates (H&E, 200×). (c) The control group showed no collagen deposition (MTC, 200×). (d) The ISO group showed marked disturbed and fragmented myocardial fibers and edema (H&E, 200×). (e) The ISO group showed marked inflammation (H&E 200×). (f) The ISO group showed marked perivascular collagen deposition (MTC 200×). (g) The DAPA + ISO group showed mild myocardial damage and edema (H&E, 200×). (h) The DAPA + ISO group showed minimal inflammation in myocardial fibers (H&E, 200×). (i) The DAPA + ISO group showed mild perivascular collagen deposition (MTC, 200×)

Table 2: Comparison between the histopathological changes in the studied groups

Variables	Control (%)	ISO group (%)	ISO + DAPA (%)	p
Degenerated muscle				
Absent	10 (100)	0	0	p1 < 0.001
Mild	0	0	10 (100)	p2 < 0.001
Moderate	0	2 (20)	0	p3 = 0.003
Marked	0	8 (80)	0	
Interstitial hemorrhage and congestion				
Absent	10 (100)	0	0	p1 < 0.001
Mild	0	0	6 (60)	p2 < 0.001
Moderate	0	9 (90)	4 (40)	p3 = 0.012
Marked	0	1 (10)	0	
Inflammation				
Absent	10 (100)	0	0	p1 < 0.001
Mild	0	0	10 (100)	p2 < 0.001
Moderate	0	9 (90)	0	p3 < 0.001
Marked	0	1 (10)	0	

Data were expressed as frequency (%) and analyzed using Monte Carlo test at p < 0.05. p1 control regarding ISO. p2 control regarding ISO + DAPA. p3 ISO regarding ISO + DAPA. DAPA: Dapagliflozin, ISO: Isoproterenol.

Immunohistochemical results

A significant SGLT2 overexpression was observed in the ISO group compared with the control group (p < 0.001). The expression was significantly decreased in the ISO + DAPA-treated group compared with the ISO group (p < 0.001; Figure 5a-c).

The SIRT1 and FASN expressions as modulators of fatty acid oxidation and autophagy were also assessed. SIRT1 nuclear expression declined at a nonsignificant level (p = 0.427) in the ISO group compared with the control group. However, SIRT1 was overexpressed in the ISO + DAPA group compared with the control and ISO groups (p = 0.047 and p < 0.001, respectively; Figure 5d-f).

Similarly, FASN expression followed a similar SIRT1 expression manner in all studied groups. FASN expression was declined at a nonsignificant level (p = 0.452) in the ISO group compared with the control group. However, FASN was overexpressed in the ISO + DAPA group compared with the control and ISO groups (p = 0.013 and p < 0.001, respectively; Figure 5g-i). Table 3 illustrated the detailed markers expression.

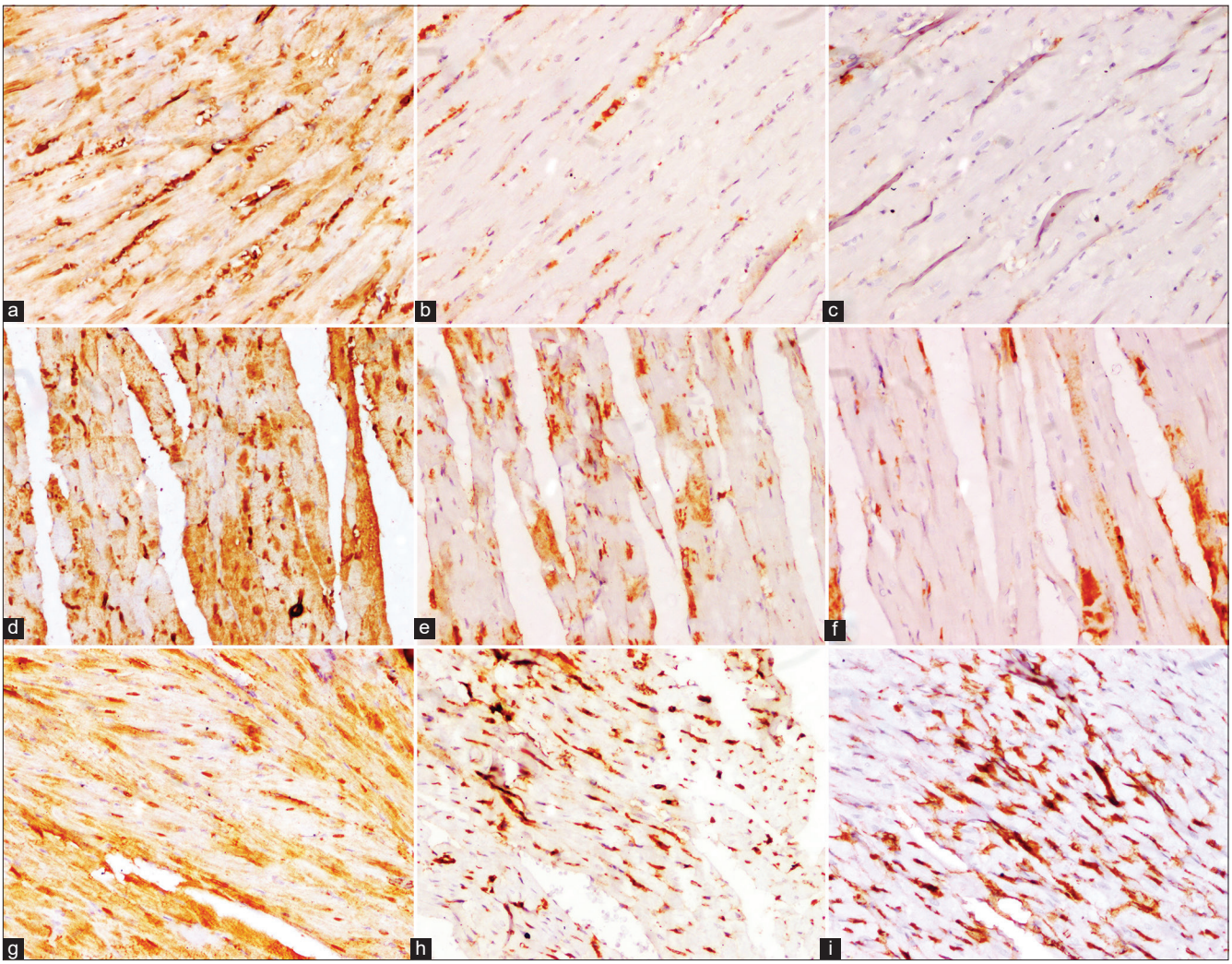

Figure 5: Immunohistochemical expression of SGLT2, SIRT1, and FASN. (a) The healthy control group showed low SGLT2. (b) SGLT2 overexpression was found in the ISO group. (c) SGLT2 expression decreased in the ISO + DAPA-treated group. (d) The healthy control group showed low SIRT1. (e) Decreased SIRT1 was observed in the ISO group. (f) The SIRT1 overexpression in the ISO + DAPA-treated group was observed. (g) The healthy control group showed low FASN. (h) Decreased FASN was observed in the ISO group. (i) FASN overexpression was observed in the ISO + DAPA-treated group, IHC, 200X

Table 3: Comparison between sodium-glucose transporter 2, silent information regulator 1, and fatty acid synthase immunohistochemical expression in the studied groups

Studied markers	Control	ISO	ISO+DAPA	p
SGLT2	14.50 ± 3.45	86.00 ± 12.53	27.50 ± 5.01	p1 < 0.001 p2 = 1.000 p3 < 0.001
SIRT1	77.50 ± 16.16	62.50 ± 8.92	131.50 ± 19.03	p1 = 0.427 p2 = 0.047 p3 < 0.001
FASN	84.50 ± 14.38	71.50 ± 8.88	99.00 ± 26.29	p1 = 0.452 p2 = 0.013 p3 < 0.001

Data were expressed as mean ± SEM and analyzed using one-way ANOVA followed by Bonferroni's *post hoc* test at $p < 0.05$. n = 10. p1 control regarding ISO. p2 control regarding ISO+DAPA. p3 ISO regarding ISO + DAPA. SGLT2: Sodium-glucose transporter 2, SIRT1: Silent information regulator 1, FASN: Fatty acid synthase, ANOVA: Analysis of variance, DAPA: Dapagliflozin, ISO: Isoproterenol, SEM: Standard error of mean.

Discussion

DAPA, a selective SGLT2 inhibitor, is reported to be a possible adjuvant therapy in HF treatment [26]. The previous studies reported the SGLT2 inhibitors' therapeutic effect in MI despite the absence of known heart receptors [27]. Therefore, identifying the possible mechanism by which DAPA protects against MI progress is an ongoing focus.

We adopted the ISO-induced MI model due to the comparable pathophysiologic and morphologic alterations of this non-invasive model to those of actual heart disorders [28]. ISO-induced MI as evidenced by a significant decrease in BP, ECG changes, and elevation of cardiac enzymes in addition to diffuse histopathological changes in the cardiac tissues. Balea *et al*. found a significant ISO role in lowering the BP in acute administration, and prolonged administration increases the BP [20]. The ECG alterations in the ISO group, reduced RR interval, increased HR, and prolonged QRS complex could result from the direct agonist effect of ISO with β1 and β2 adrenoreceptor, slowing of ventricular conduction, and subendocardial ischemia and necrosis [20], [29]. In addition, ST depression could result from the loss of action potential in the myocardial cell membrane induced by ROS overproduction and oxidative stress [20]. Furthermore, ISO induced cardiac damage with the release of cytosolic destructive enzymes [30].

We observed different mechanism by which ISO-induced acute MI picture by altering different mechanisms. ISO altered ROS production and the release of the inflammatory mediators. The ROS alterations in the cardiac tissue homogenates, increased MDA, and decreased SOD and GSH could participate in cardiac damage through the depletion of the antioxidant enzymes and mitochondrial dysfunction [31], [32]. Moreover, the activation of the inflammatory cytokines (e.g., TNF-α and IL-6) induced myocardial inflammation and subsequent fibrosis [33]. ISO induced MI by altering the lipid profile with the elevation of cholesterol and TG levels, the key risk factors in the development of coronary artery diseases [34].

DAPA pre-treated rats showed a significant reversal of ISO-induced MI changes. SBP improvement, lack of ECG alterations, restoration of muscle integrity with normalization of cardiac enzymes level, reduction of ROS levels and inflammatory mediators, and dramatic improvement in the histopathological changes were significantly observed. The previous studies reported a possible modulatory role of DAPA in enhancing cardiac hemodynamic and cell integrity, reducing free radical levels, and inhibiting lipid peroxidation [35], [36], [37], [38], [39]. The cardioprotective activity of DAPA could be directly induced through the stabilization of the cardiac cells by modulating sodium influx and Ca^{2+} homeostasis [40]. In addition, DAPA could inhibit the production of different pro-inflammatory cytokines [41]. DAPA could indirectly induced cardioprotection effect by lowering serum cholesterol and TG levels, the common risk factors for vascular endotheliitis and cardiac fibrosis [42].

Despite the previously reported DAPA mechanisms, ongoing studies are on focus to identify the mechanism by which SGLT2 inhibitor protects against acute MI. Kashiwagi *et al*. reported a definite SGLT1 expression as a cardioprotective mechanism in low-glucose heart disease, but SGLT2 expression has not yet been well-defined [43]. Furthermore, the previous studies reported the absence of SGLT2 expression in either human or adult rat heart, excluding a direct SGLT2 inhibition action [44]. The relatively low SGLT2 expression in other studies could result from a crosstalk between the antibody and other SGLT isoforms normally expressed in heart tissue [44].

In the present study, SGLT2 expression was low in the cardiac muscle of the healthy control group, but the expression was elevated in the ISO group. Moreover, the SGLT2 expression markedly declined to reach an almost normal level in the ISO + DAPA group. Lee *et al.* found a transient SGLT2 elevation in the acute MI limited to the infarction area while the level diminished with the chronicity of the disease [45]. Furthermore, Lee *et al.* explained the negative SGLT2 expression in the study by Di Franco due to the inadequacy of the experimental time required for the up- or down-regulation of proteins [46].

We further studied the SIRT1/FASN and SGLT2 expressions in untreated and treated MI groups. SIRT1 was expressed at a low level in the cardiac muscle, which is consistent with its physiological function. SIRT1 protects against aging and apoptosis by controlling the expression of several proteins responsive to oxidative stress [47]. However, the expression was not significantly declined in MI contrary to the previous studies [48]. The previous studies were conducted in diabetic mouse models. Diabetes impaired mitochondrial and peroxisomal stability, promoted the formation of unfolded proteins, and suppressed autophagy [49]. In addition, the discrepancy could be

due to the acute disease onset in our study in contrast to the chronic heart disease model in the previous studies.

The overexpression of SIRT1 in the ISO + DAPA-treated group could be result from a crosstalk between SGLT2 inhibitor and SIRT1. SIRT1 mediated the SGLT2i function through the activation of hypoxia-induced factor-2 alpha (HIF-2α). HIF-2α is the initial regulator for erythropoietin synthesis, the most powerful predictor of lowering the risk of HF in clinical trials [49]. SIRT1 activation promoted autophagy to alleviate oxidative stress and prevent cardiac injury in type 2 DM [50]. Moreover, SGLT2 inhibitors promoted ketogenic nutrient deprivation that induced SIRT1 activation and cardioprotection against HF [38].

In the present study, FASN expression followed the expression of SIRT1 in cardiac tissues. SIRT1 induced FASN expression to maintain lipid and glucose metabolism through the regulation of different mechanisms [51], [52]. However, the beneficial FASN role in cardiac disease is a matter of controversy. FASN induction in stressed myocardium represents a compensatory response to protect cardiomyocytes from pathological calcium flux [53]. On the contrary, FASN expression could be associated with decreased cardiac output and impaired oxygen supply, leading to HF [54]. A study performed by Hansmann *et al*. found that peroxisome proliferator-activated receptor-gamma activation triggered the FASN upregulation and signs of HF within 2 months [55]. Abd Alla *et al*. concluded that initial FASN upregulation may be beneficial by supplying more energy substrate to the heart muscle whereas long-term exposure may induce cardiomyopathy and HF [56]. Therefore, further studies are required to validate the functional role of FASN activation in heart tissue on short- and long-term activation. This could necessitate the usage of combinational SGLT2 and FASN inhibitors in MI prevention and subsequent HF.

Conclusion

Pre-treatment with SGLT2i (DAPA) may reduce the effects of ISO-induced oxidative stress, elevation of inflammatory cytokines (TNF- and IL-6), and overactivity of myocardial sympathetic flow, which raises the oxygen demand of the cardiac muscles. Although SGLT2 expression is modest in healthy cardiac muscle, acute MI may enhance its expression. The regulation of SIRT1 and FASN expression in the cardiac muscle may be the mechanism by which DAPA exerts its potential cardioprotective effect against MI.

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References

1. Rastogi A, Novak E, Platts AE, Mann DL. Epidemiology, pathophysiology and clinical outcomes for heart failure patients with a mid-range ejection fraction. Eur J Heart Fail. 2017;19(12):1597-605. https://doi.org/10.1002/ejhf.879
   PMid:29024350
2. Adnan G, Singh DP, Mahajan K. Coronary Artery Thrombus. Treasure Island, FL: StatPearls; 2021.
3. Ojha N, Dhamoon AS. Myocardial Infarction. Treasure Island, FL: StatPearls Publishing; 2021.
4. Sharifi-Rad M, Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, *et al*. Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. Front Physiol. 2020;11:694. https://doi.org/10.3389/fphys.2020.00694
   PMid:32714204
5. D'Onofrio N, Servillo L, Balestrieri ML. SIRT1 and SIRT6 signaling pathways in cardiovascular disease protection. Antioxid Redox Signal. 2018;28(8):711-32. https://doi.org/10.1089/ars.2017.7178
   PMid:28661724
6. Hashimoto H, Olson EN, Bassel-Duby R. Therapeutic approaches for cardiac regeneration and repair. Nat Rev Cardiol. 2018;15(10):585-600. https://doi.org/10.1038/s41569-018-0036-6
   PMid:29872165
7. Brown E, Wilding JP, Alam U, Barber TM, Karalliedde J, Cuthbertson DJ. The expanding role of SGLT2 inhibitors beyond glucose-lowering to cardiorenal protection. Ann Med. 2020;53(1):2072-89. https://doi.org/10.1080/07853890.2020.1841281
   PMid:33107349
8. Sano R, Shinozaki Y, Ohta T. Sodium-glucose cotransporters: Functional properties and pharmaceutical potential. J Diabetes Investig. 2020;11(4):770-82. https://doi.org/10.1111/jdi.13255
   PMid:32196987
9. Hsia DS, Grove O, Cefalu WT. An update on sodium-glucose co-transporter-2 inhibitors for the treatment of diabetes mellitus. Curr Opin Endocrinol Diabetes Obes. 2017;24(1):73-9. https://doi.org/10.1097/MED.0000000000000311
   PMid:27898586
10. Ni L, Yuan C, Chen G, Zhang C, Wu X. SGLT2i: Beyond the glucose-lowering effect. Cardiovasc Diabetol. 2020;19(1):98. https://doi.org/10.1186/s12933-020-01071-y
    PMid:32590982
11. Maejima Y. SGLT2 inhibitors play a salutary role in heart failure via modulation of the mitochondrial function. Front Cardiovasc Med. 2020;6:186. https://doi.org/10.3389/fcvm.2019.00186
    PMid:31970162
12. Ren Z, He H, Zuo Z, Xu Z, Wei Z, Deng J. The role of different SIRT1-mediated signaling pathways in toxic injury. Cell Mol Biol Lett. 2019;24:36. https://doi.org/10.1186/s11658-019-0158-9

PMid:31164908

13. Swe MT, Thongnak L, Jaikumkao K, Pongchaidecha A, Chatsudthipong V, Lungkaphin A. Dapagliflozin not only improves hepatic injury and pancreatic endoplasmic reticulum stress, but also induces hepatic gluconeogenic enzymes expression in obese rats. Clin Sci (Lond). 2019;133(23):2415-30. https://doi.org/10.1042/CS20190863
PMid:31769484
14. Fhu CW, Ali A. Fatty acid synthase: An emerging target in cancer. Molecules. 2020;25(17):3935. https://doi.org/10.3390/molecules25173935
PMid:32872164
15. Jou J, Choi SS, Diehl AM. Mechanisms of disease progression in nonalcoholic fatty liver disease. Semin Liver Dis. 2008;28(4):370-9. https://doi.org/10.1055/s-0028-1091981
PMid:18956293
16. He Y, Huang W, Zhang C, Chen L, Xu R, Li N, *et al*. Energy metabolism disorders and potential therapeutic drugs in heart failure. Acta Pharm Sin B. 2021;11(5):1098-116. https://doi.org/10.1016/j.apsb.2020.10.007
PMid:34094822
17. Sert NP, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, *et al*. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. Br J Pharmacol. 2020;177(16):3617-24. https://doi.org/10.1111/bph.15193
PMid:32662519
18. Huang H, Geng Q, Yao H, Shen Z, Wu Z, Miao X, *et al*. Protective effect of scutellarin on myocardial infarction induced by isoprenaline in rats. Iran J Basic Med Sci. 2018;21(3):267-76. https://doi.org/10.22038/ijbms.2018.26110.6415
PMid:29511493
19. Fedorova OV, Grigorova Y, Hagood M, Long J, McDevitt R, McPherson R, *et al*. P3□209: Cognitive impairement is associated with premature arterial stiffening, aortic wall fibrosis and increased blood pressure: A novel rat model of age-dependent vascular dementia. Alzheimers Dement. 2018;14(7S_Part_21):P1149-50. https://doi.org/10.1016/j.jalz.2018.06.1568
20. Balea ŞS, Pârvu AE, Pop N, Marín FZ, Pârvu M. Polyphenolic compounds, antioxidant, and cardioprotective effects of pomace extracts from fetească neagră cultivar. Oxid Med Cell Longev. 2018;2018:8194721. https://doi.org/10.1155/2018/8194721
PMid:29765504
21. Konopelski P, Ufnal M. Electrocardiography in rats: A comparison to human. Physiol Res. 2016;65(5):717-25. https://doi.org/10.33549/physiolres.933270
PMid:27429108
22. Stevens AB. Theory and Practice of Histological Techniques. London: Churchill Livingstone; 1996.
23. Wu X, Zayzafoon M, Zhang X, Hameed O. Is there a role for fatty acid synthase in the diagnosis of prostatic adenocarcinoma? A comparison with AMACR. Am J Clin Pathol. 2011;136(2):239-46. https://doi.org/10.1309/AJCP0Y5QWWYDKCJE
PMid:21757596
24. Bai W, Zhang X. Nucleus or cytoplasm? The mysterious case of SIRT1's subcellular localization. Cell Cycle. 2016;15(24):3337-8. https://doi.org/10.1080/15384101.2016.1237170
PMid:27687688
25. Kuang H, Liao L, Chen H, Kang Q, Shu X, Wang Y. Therapeutic effect of sodium glucose co-transporter 2 inhibitor dapagliflozin on renal cell carcinoma. Med Sci Monit. 2017;23:3737-45. https://doi.org/10.12659/msm.902530
PMid:28763435
26. McEwan P, Darlington O, McMurray JJ, Jhund PS, Docherty KF, Böhm M, *et al*. Cost-effectiveness of dapagliflozin as a treatment for heart failure with reduced ejection fraction: A multinational health-economic analysis of DAPA-HF. Eur J Heart Fail. 2020;22(11):2147-56. https://doi.org/10.1002/ejhf.1978
PMid:32749733
27. Li X, Lu Q, Qiu Y, Do Carmo JM, Wang Z, Da Silva AA, *et al*. Direct cardiac actions of the sodium glucose co-transporter 2 inhibitor empagliflozin improve myocardial oxidative phosphorylation and attenuate pressure-overload heart failure. J Am Heart Assoc. 2021;10(6):e018298. https://doi.org/10.1161/JAHA.120.018298
PMid:33719499
28. Li X, Zhang ZL, Wang HF. Fusaric acid (FA) protects heart failure induced by isoproterenol (ISP) in mice through fibrosis prevention via TGF-β1/SMADs and PI3K/AKT signaling pathways. Biomed Pharmacother. 2017;93:130-45. https://doi.org/10.1016/j.biopha.2017.06.002
PMid:28624424
29. Mustroph J, Wagemann O, Lücht CM, Trum M, Hammer KP, Sag CM, *et al*. Empagliflozin reduces Ca/calmodulin-dependent kinase II activity in isolated ventricular cardiomyocytes. ESC Heart Fail. 2018;5(4):642-8. https://doi.org/10.1002/ehf2.12336
PMid:30117720
30. Chengji W, Xianjin F. Exercise protects against diabetic cardiomyopathy by the inhibition of the endoplasmic reticulum stress pathway in rats. J Cell Physiol. 2019;234(2):1682-8. https://doi.org/10.1002/jcp.27038
PMid:30076729
31. Tian L, Cao W, Yue R, Yuan Y, Guo X, Qin D, *et al*. Pretreatment with tilianin improves mitochondrial energy metabolism and oxidative stress in rats with myocardial ischemia/reperfusion injury via AMPK/SIRT1/PGC-1 alpha signaling pathway. J Pharmacol Sci. 2019;139(4):352-60. https://doi.org/10.1016/j.jphs.2019.02.008
PMid:30910451
32. Khodir SA, Sweed E, Gadallah M, Shabaan A. Astaxanthin attenuates cardiovascular dysfunction associated with deoxycorticosterone acetate-salt-induced hypertension in rats. Clin Exp Hypertens. 2022;44(4):1-14. https://doi.org/10.1080/10641963.2022.2055764
PMid:35322744
33. Yang L, Wang B, Zhou Q, Wang Y, Liu X, Liu Z, *et al*. MicroRNA-21 prevents excessive inflammation and cardiac dysfunction after myocardial infarction through targeting KBTBD7. Cell Death Dis. 2018;9(7):769. https://doi.org/10.1038/s41419-018-0805-5
PMid:29991775
34. Rajadurai M, Prince PS. Preventive effect of naringin on cardiac markers, electrocardiographic patterns and lysosomal hydrolases in normal and isoproterenol-induced myocardial infarction in Wistar rats. Toxicology. 2007;230(2-3):178-88. https://doi.org/10.1016/j.tox.2006.11.053
PMid:17188415
35. Andreadou I, Efentakis P, Balafas E, Togliatto G, Davos CH, Varela A, *et al*. Empagliflozin limits myocardial infarction *in vivo* and cell death *in vitro*: Role of STAT3, mitochondria, and redox aspects. Front Physiol. 2017;8:1077. https://doi.org/10.3389/fphys.2017.01077
PMid:29311992
36. Pruett JE, Fernandez ED, Everman SJ, Vinson RM, Davenport K, Logan MK, *et al*. Impact of SGLT-2 inhibition on cardiometabolic abnormalities in a rat model of polycystic ovary syndrome. Int J Mol Sci. 2021;22(5):2576. https://doi.org/10.3390/ijms22052576
PMid:33806551
37. Zhang Y, Liu Z, Zhou M, Liu C. Therapeutic effects of fibroblast growth factor21 against atherosclerosis via the NFκB pathway.

Mol Med Rep. 2018;17(1):1453-60. https://doi.org/10.3892/mmr.2017.8100

PMid:29257234

38. Packer M. Cardioprotective effects of sirtuin-1 and its downstream effectors: Potential role in mediating the heart failure benefits of SGLT2 (sodium-glucose cotransporter 2) inhibitors. Circ Heart Fail. 2020;13(9):e007197. https://doi.org/10.1161/CIRCHEARTFAILURE.120.007197

PMid:32894987

39. Rezq S, Nasr AM, Shaheen A, Elshazly SM. Doxazosin down-regulates sodium-glucose cotransporter-2 and exerts a renoprotective effect in rat models of acute renal injury. Basic Clin Pharmacol Toxicol. 2020;126(5):413-23. https://doi.org/10.1111/bcpt.13371

PMid:31788938

40. Uthman L, Baartscheer A, Bleijlevens B, Schumacher CA, Fiolet JW, Koeman A, *et al*. Class effects of SGLT2 inhibitors in mouse cardiomyocytes and hearts: Inhibition of Na^+/H^+ exchanger, lowering of cytosolic Na^+ and vasodilation. Diabetologia. 2018;61(3):722-6. https://doi.org/10.1007/s00125-017-4509-7

PMid:29197997

41. Hussein AM, Eid EA, Taha M, Elshazli RM, Bedir RF, Lashin LS. Comparative study of the effects of GLP1 analog and SGLT2 inhibitor against diabetic cardiomyopathy in Type 2 diabetic rats: Possible underlying mechanisms. Biomedicines. 2020;8(3):43. https://doi.org/10.3390/biomedicines8030043

PMid:32106580

42. Arow M, Waldman M, Yadin D, Nudelman V, Shainberg A, Abraham NG, *et al*. Sodium-glucose cotransporter 2 inhibitor dapagliflozin attenuates diabetic cardiomyopathy. Cardiovasc Diabetol. 2020;19(1):7. https://doi.org/10.1186/s12933-019-0980-4

PMid:31924211

43. Kashiwagi Y, Nagoshi T, Yoshino T, Tanaka TD, Ito K, Harada T, *et al*. Expression of SGLT1 in human hearts and impairment of cardiac glucose uptake by phlorizin during ischemia-reperfusion injury in mice. PLoS One. 2015;10(6):e0130605. https://doi.org/10.1371/journal.pone.0130605

PMid:26121582

44. Van Steenbergen A, Balteau M, Ginion A, Ferté L, Battault S, Ravenstein CM, *et al*. Sodium-myoinositol cotransporter-1, SMIT1, mediates the production of reactive oxygen species induced by hyperglycemia in the heart. Sci Rep. 2017;7:41166. https://doi.org/10.1038/srep41166

PMid:28128227

45. Lee SY, Lee TW, Park GT, Kim JH, Lee HC, Han JH, *et al*. Sodium/glucose co-transporter 2 inhibitor, empagliflozin, alleviated transient expression of SGLT2 after myocardial infarction. Korean Circ J. 2021;51(3):251-62. https://doi.org/10.4070/kcj.2020.0303

PMid:33655725

46. Di Franco A, Cantini G, Tani A, Coppini R, Zecchi-Orlandini S, Raimondi L, *et al*. Sodium-dependent glucose transporters (SGLT) in human ischemic heart: A new potential pharmacological target. Int J Cardiol. 2017;243:86-90. https://doi.org/10.1016/j.ijcard.2017.05.032

PMid:28526540

47. Alcendor RR, Gao S, Zhai P, Zablocki D, Holle E, Yu X, *et al*. Sirt1 regulates aging and resistance to oxidative stress in the heart. Circ Res. 2007;100(10):1512-21. https://doi.org/10.1161/01.RES.0000267723.65696.4a

PMid:17446436

48. Lu TM, Tsai JY, Chen YC, Huang CY, Hsu HL, Weng CF, *et al*. Downregulation of sirt1 as aging change in advanced heart failure. J Biomed Sci. 2014;21(1):57. https://doi.org/10.1186/1423-0127-21-57

PMid:24913149

49. Packer M. Autophagy-dependent and independent modulation of oxidative and organellar stress in the diabetic heart by glucose-lowering drugs. Cardiovasc Diabetol. 2020;19(1):62. https://doi.org/10.1186/s12933-020-01041-4

PMid:32404204

50. Ma S, Feng J, Zhang R, Chen J, Han D, Li X, *et al*. SIRT1 activation by resveratrol alleviates cardiac dysfunction via mitochondrial regulation in diabetic cardiomyopathy mice. Oxid Med Cell Longev. 2017;2017:4602715. https://doi.org/10.1155/2017/4602715

PMid:28883902

51. Majeed Y, Halabi N, Madani AY, Engelke R, Bhagwat AM, Abdesselem H, *et al*. SIRT1 promotes lipid metabolism and mitochondrial biogenesis in adipocytes and coordinates adipogenesis by targeting key enzymatic pathways. Sci Rep. 2021;11(1):8177. https://doi.org/10.1038/s41598-021-87759-x

PMid:33854178

52. Choi WI, Yoon JH, Choi SH, Jeon BN, Kim H, Hur MW. Proto-oncoprotein Zbtb7c and SIRT1 repression: Implications in high-fat diet-induced and age-dependent obesity. Exp Mol Med. 2021;53(5):917-32. https://doi.org/10.1038/s12276-021-00628-5

53. Razani B, Zhang H, Schulze PC, Schilling JD, Verbsky J, Lodhi IJ, *et al*. Fatty acid synthase modulates homeostatic responses to myocardial stress. J Biol Chem. 2011;286(35):30949-61. https://doi.org/10.1074/jbc.M111.230508

PMid:21757749

54. Abdalla S, Fu X, Elzahwy SS, Klaetschke K, Streichert T, Quitterer U. Up-regulation of the cardiac lipid metabolism at the onset of heart failure. Cardiovasc Hematol Agents Med Chem. 2011;9(3):190-206. https://doi.org/10.2174/187152511797037583

PMid:21711241

55. Hansmann G, Wagner RA, Schellong S, Perez VA, Urashima T, Wang L, *et al*. Pulmonary arterial hypertension is linked to insulin resistance and reversed by peroxisome proliferator-activated receptor-gamma activation. Circulation. 2007;115(10):1275-84. https://doi.org/10.1161/CIRCULATIONAHA.106.663120

PMid:17339547

56. Alla JA, Graemer M, Fu X, Quitterer U. Inhibition of G-protein-coupled receptor kinase 2 prevents the dysfunctional cardiac substrate metabolism in fatty acid synthase transgenic mice. J Biol Chem. 2016;291(6):2583-600. https://doi.org/10.1074/jbc.M115.702688

PMid:26670611