Dapagliflozin Ameliorates Myocardial Remodeling in Heart Failure Induced Diabetic Rats

Soad L. Kabil [1], Nabila H. Fahmy [1], Doaa S. Fadaly [1], Nevertyty M. Mahmoud [1]
[1]Clinical Pharmacology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt.

Abstract

Background: Diabetes mellitus is an independent risk factor for heart failure (HF). HF is the leading cause of hospitalization in type 2 diabetes (T2D) patients. Dapagliflozin is a highly selective sodium-glucose cotransporter-2 inhibitor that was recently developed to treat T2D. Dapagliflozin was found to reduce the incidence of cardiovascular mortality and HF hospitalisation. Dapagliflozin has been approved for the treatment of HF with reduced ejection fraction (HFrEF) patients by FDA in 2020. However, the mechanisms through which dapagliflozin mediate these benefits are not completely understood and its efficacy and clinical application are still controversial.

Objectives: to investigate the effect and possible underlying mechanism of action of dapagliflozin on HF induced diabetic rats.

Methods: HF was induced in diabetic rats by ligation of the left anterior descending coronary artery. Dapagliflozin (1 mg/kg/day) was administered for four weeks through gavage. Body weight (BW), heart weight (HW), heart rate (HR), blood pressure, fasting serum glucose, insulin, glycated hemoglobin A1C (HbA1c), cardiac specific enzymes [creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), cardiac troponin I (cTnI)], fibrotic markers [procollagen I C-terminal propeptide (PICP), procollagen III N-terminal peptide (PIIINT), transforming growth factor beta 1 (TGFβ 1), inflammatory mediators [tumor necrosis factor-alpha (TNF-α)], anti-oxidative markers [malondialdehyde (MDA) and super oxide dismutase (SOD)] and apoptotic mediators (BCL2 and caspase 3) were measured. Histopathological examination using hematoxylin and eosin for heart, lung and liver and masson trichrome for cardiac tissue was done. Vascular reactivity and direct effect of dapagliflozin on myocardial contractility was evaluated.

Results: Dapagliflozin decreased blood glucose, HW, HW/BW, CK-MB, LDH, cTn1, PICP, PIIINP, TGF-β1, TNF-α, MDA and cardiac level of Caspase 3, while increased SOD and BCL2 levels. Dapagliflozin improved the histopathological picture of heart, liver and lung and improved the vascular reactivity of the aorta in HF induced diabetic rats.

Conclusion: Dapagliflozin alleviates myocardial remodeling and dysfunction in HF induced diabetic rats by attenuating hyperglycemia, fibrosis, inflammation, oxidative stress and apoptosis. Dapagliflozin improved histo-pathological changes in heart, lung and liver and improved vascular reactivity.

Keywords: heart failure; type 2 diabetes; Dapagliflozin; fibrosis; inflammation; apoptosis; vascular reactivity.

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Introduction

Diabetes mellitus (DM) is a major health problem **(Bahar et al., 2020).** T2D accounts for 90–95% of all diagnosed diabetes in adults and still growing globally **(Mayer-Davis et al., 2017).** DM is considered an independent risk factor for cardiovascular disease (CVD) **(Kenny and Abel, 2019)**. Diabetic patients have a CVD risk that is comparable to that of patients who have had a previous myocardial infarction (MI) **(Schramm et al., 2008; Russell and Cooper 2015).** Peripheral arterial disease (16.2 %) and HF (14.7 %) are the most common early presentations of CVD in diabetic patients, followed by angina and non-fatal MI **(Shah et al., 2015).** Also, Diabetic patients are more than twice as likely as non-diabetic patients to develop HF (**Dei Cas et al., 2015).**

HF is a prevalent condition among T2D patients, with an elevated risk of mortality and morbidity **(pham et al., 2017)**. While 10-15% of diabetic patients have HF, a recent study suggested that 44% of patients hospitalized for HF have diabetes **(Echouffo-Tcheugui et al., 2016).** It's challenging to achieve optimal glycemic control in this patient because several available therapies can aggravate HF symptoms. **(Singh et al., 2016).** Besides, until recently, no anti-hyperglycemic therapies for T2D have been found to improve HF outcomes **(pham et al., 2017).**

Inhibitors of sodium- glucose cotransporter (SGLT) 2 are a new class of oral drugs for patients with T2D which show positive effect on glucose control **(Han et al., 2015).** Dapagliflozin, is a highly selective and reversible SGLT2 inhibitor approved for the treatment of T2D **(Fioretto et al., 2015; Kosiborod et al., 2017).** Adding SGLT2 inhibitors, specifically dapagliflozin, to the standard of therapy has been shown to diminish cardiovascular and microvascular complications associated with T2D **(Dziuba et al., 2014; Kosiborod et al., 2017).** Dapagliflozin also has been proven to have a beneficial effect on patients with HF in addition to improving hyperglycemia **(Petrie et al., 2020; Kosiborod et al., 2020)**. The FDA has approved dapagliflozin in 2020 for the treatment of adult patients with HFrEF (with or without T2D) to reduce the risk of cardiovascular death and hospitalization for HF **(Murphy et al., 2020)**. However, the mechanisms through which SGLT2 inhibitors mediate these benefits are not understood **(Kenny and Abel, 2019)** and their efficacy and clinical application are still controversial, with no standardized clinical guidelines for reference (**Cai et al., 2021)**. Consequently, the present study aimed to investigate the effect and possible underlying mechanism of action of dapagliflozin on HF induced diabetic rats.

Materials and methods

- Drugs and Chemicals

Dapagliflozin was purchased from (AstraZeneca Co, Ltd), Streptozotocin (Sigma Company, England), Nicotinamide (E.I.P.I.Co.A.R.E), Phenyle ephrine and acetylcholine powder (Sigma company, England), Isoflurane solution (sigma company, Egypt), Pentobarbitone sodium solution (CEVA, Egypt), heparin sodium (Rockhardt UK Ltd, Wrexham, UK), Penicillin G vials: (CID Co. for Medical industries, Egypt), Haematoxlin and Eosin (H & E) (Sigma, St. Louis, MO., USA), Masson's Trichrome Stain Kit (Polysciences, Inc., USA), Formalin 10% (El Nasr pharmaceutical company, Egypt), Liquid Nitrogen (Al Gomhuria Company, Egypt).

- Animals

24 male wistar rats (8–10 weeks old, 200-250 gm/each) were purchased from Faculty of Veterinary Medicine's animal unit, Zagazig University. All experimental procedures were approved by The Institutional Animal Care and Use Committee Zagazig University (ZU-IACUC) "No. ZU-IACUC/3/F/56/2018" and it was consistent with the National Institutes of Health guides for care and use of laboratory animals (NIH publications) "No.8023, revised 1996". Before starting the study, rats were given 1 week to acclimate in their cages. Rats had freely standard chow and water in a controlled temperature of 23 ± 2 °C with a 12 hour light–dark cycle.

Induction of T2D

Rats received single intraperitoneal injection (I.P) of 55 mg/kg streptozotocin (STZ) in fresh buffer solution preceded by an I.P injection of nicotinamide at a dose of 270/mg/kg **(Szkudelski, 2012),** oral glucose was administrated for 24 hours to prevent hypoglycemia. The fasting blood glucose levels of the rats in the experimental group was estimated after one week of injection, the rats with fasting blood glucose levels ≥ 160 mg/dl was considered diabetic **(Gandhi et al., 2013**).

Induction of HF

A left thoracotomy was performed after rats were exposed to 1.5–2% isoflurane supplemented with 100% oxygen. The left anterior descending (LAD) coronary artery was occluded using a 6-0 polypropylene suture and the thorax was immediately closed **(Saraiva et al., 2007).** Sham group was exposed to the same surgical procedure without LAD coronary artery ligation. Following MI surgery, the rats took 6 weeks to recover and develop the HF state **(Pfeffer et al., 1979).** For conformation of occurrence of myocardial dysfunction and HF, serum level of CK-MB was estimated for all operated rats. Rats which have significant increase in CK-MB suggested successful construction of HF model **(Li et al., 2021; Peng et al., 2021).**

Experimental design

24 male wistar rats were randomly divided into 4 groups (6 rats / each). *Control normal group* ***(CN),*** *diabetic sham group* ***(DM sham),*** *diabetic HF group* ***(DM.HF)*** ,all of them received the vehicle throughout the experiment, and *diabetic HF dapagliflozin treated group* ***(DM.HF.dapa)***, received dapagliflozin (1 mg/ kg/ day) dissolved in distilled water by gavage for 4 weeks **(Tanajak, 2018; Gong et al., 2021).** For appropriate follow-up and dosages determination, rats were weighed daily. Dapagliflozin treatment was discontinued around 24 h before the end of the study to stop its pharmacological effects.

At the end of the experimental period; BW (measured by *digital weight scale*), blood pressure and ECG of the rats were assessed by *Power Lab (4/35) data acquisition system* (Australia). After that upon excision of the heart, whole blood was aspirated (3-5mL) from the carotid artery in clean dry test tubes through rat arterial polyethylene cannula, and then the animals were euthanized (Exsanguination/cardiac perfusion under anesthesia). The serum was separated by centrifugation at 3000 rpm for 10 minutes and the obtained serum was stored rapidly at - 80°c after being divided into several fractions for later bioassay of fasting serum glucose, insulin, HbA1c, cardiac-Specific enzymes (CK-MB, LDH and TnI), markers of cardiac remodeling and fibrosis (PICP, PIIINT and TGFβ

1), inflammatory mediator (TNF-α), oxidative stress mediators (MDA and SOD) and apoptotic marker (Bcl-2).

After that hearts were excised, rinsed carefully with saline then dried and weighted to assess HW/BW by the following equation [heart weight body weight ratio = heart wt. / body wt. × 100], then hearts was divided into two portions one part was preserved in formalin 10% for histopathological examination and the second part was frozen by liquid nitrogen and then stored till homogenized at -80°c for biochemical determination of caspase 3. The lungs and livers also were excised and preserved in formalin 10% for histopathological examination. Thoracic aorta was obtained for the evaluation of vascular reactivity.

- **Assay of fasting serum glucose**: The fasting serum glucose levels were assayed by kits supplied as (Diamond Diagnostics, Egypt) according to the method adopted by **Trinder (1969)**.
- **Assay of serum insulin:** serum insulin levels were measured by rat insulin Elisa kits according to manufacturer's instruction (Catalog No. 90010, *crystal chem, USA*).
- **Assay of HbA1c:** HbA1c serum levels were measured by colorimetric enzymatic assay **method** using Rat HbA1c Assay Kits (Catalog No. 80300, *Crystal Chem, USA*).
- **Assay of CK-MB:** CK-MB serum levels were assayed by the method adopted by **Szasz et al., 1976** using CK-MB kits *(BioMed diagnostics, Germany)*.
- **Assay of LDH:** LDH serum levels were assayed by the method adopted by **Vassault et al., (1986)** using LDH kits *(BioMed diagnostics, Germany)*.
- **Assay of cTnI:** cTnI serum levels were measured by a competitive Elisa method using Rat cTnI Elisa kits according to manufacturer's instruction (Catalog No. MBS727624, *MyBiosource. Inc, San Deigo, USA)*.
- **Assay of PICP:** PICP serum levels were measured by a quantitative sandwich ELISA **method** using Rat PICP Elisa Kits according to manufacturer's instruction (Catalog No. MBS2506938, *MyBiosource. Inc, San Deigo, USA*).
- **Assay of PIIINT**: PIIINT serum levels were measured by a quantitative sandwich Elisa method using Rat PIIINT ELISA Kits according to manufacturer's instruction (Catalog No. MBS163120, *MyBiosource. Inc, San Deigo, USA*).
- **Assay of TGFB1:** TGFB1 serum levels were measured by a quantitative sandwich Elisa method using Rat TGFβ 1 PicoKine ™ ELISA Kits according to manufacturer's instruction (Catalog No. MBS175833, *Boster Biological Technology, Pleasanton CA, USA*).
- **Assay of TNF-α**: TNF-α serum levels were measured by a quantitative sandwich Elisa **method** using Rat TNF-alpha ELISA Kits according to manufacturer's instruction (Catalog NO. ERA56RB, *Invitrogen California, USA)*.
- **Assay of MDA:** MDA serum levels were assayed by the method adopted by **Satoh, 1978; Ohkawa, et al., 1979** using Lipid Peroxidation MDA Assay Kits *(Bio-diagnostic* **company**, *Egypt)*.
- **Assay of SOD:** SOD serum levels were assayed by the method adopted by **Nishikimi et al., 1972** using SOD Colorimetric assay Kits *(Bio-diagnostic company, Egypt*).

- **Assay of Rat BCL-2:** BCL-2 serum levels were measured by a quantitative sandwich Elisa method using Rat BCL2 / Bcl-2 ELISA Kits according to manufacturer's instruction (Catalog No. LS-F4135, *LifeSpan BioScinces inc. North America)*).
- **Assay of tissue Caspase 3:** Caspase 3 cardiac levels were measured by a quantitative competitive Elisa method using Rat Caspase 3 ELISA Kits according to manufacturer's instruction (Catalog No. MBS743552, *MyBiosource. Inc, San Deigo, USA*).
- **Tissue *homogenates***

Tissues were rinsed in ice-cold phosphate-buffered saline (PBS) (0.02mol/L, pH 7.0-7.2) to remove excess blood carefully before homogenization. Using a glass homogenizer on ice, the tissues were minced into small pieces and were homogenised in 500ul of PBS. Then, Two freeze-thaw cycles were used to further disrupt the cell membranes in the resultant suspension. After that, the homogenates were centrifugated at 1500×g (or 5000 rpm) for 15 minutes. The supernate was removed and the samples was divided into aliquots to be stored at -80°C.

- **Histopathological *analysis***

Heart, lung and liver tissues were fixed in 10% formalin, embedded in paraffin, sectioned at 5-mm thick placed on glass slide, stained with H&E in standard histological manner and observed under light microscope to assess morphological changes. The myocyte cross-sectional area is assessed by ×400 magnification within the left ventricle. The heart was also stained with Masson trichrome.

Ex vivo experiment

- **Assessment of vascular reactivity:**

Apparatus: 10 ml capacity water jacketed automatic multi-chamber organ bath system (Palmer,England) has been used. ***Procedure:*** After measurement of the arterial blood pressure, rat's chest was opened. The aorta was cut as near the heart as possible and dissected free for long distance as possible. Thoracic aortas were rapidly placed in warm oxygenated Krebs' solution [(NaCl (6.9), KCl (0.35), KH_2PO_4 (0.16), $MgSO_4$ $7H_2O$ (0.3), $CaCl_2$ $2H_2O$ (0.37), $NaHCO_3$ (2.1) and glucose (1.05)] **(Cocks et al., 1988).** The aorta was cut into spiral shaped manner to produce contiguous strips about 4 mm wide and 3-4 cm long according to **furchgott, 1960**. A thread was attached at each end of the preparation. One end was attached to fine glass tube through which passed oxygen and the other end was attached to a side lever writing on a slowly moving smoked drum. The aorta was kept in 10 ml capacity organ bath containing Krebs solution and the temperature of the medium was kept constant at 37 °C using an electric thermostat. The preparation was then left in the bath for about 60 to 90 min to equilibrate after dissection. ***Experimental design:*** A cumulative concentration-response curve was created once the tissue had stabilised. This was accomplished by doubling the concentration of phenyl ephrine (Phe) in the preparation without washing it out, allowing enough time for each contraction to have its full effect. By increasing the agonist concentration (ranging from 10^{-7} - 10^{-4} M), the tissue was contracted step by step without relaxation and the response was recorded. The preparation was extensively rinsed after the last concentration of 10−4 M, until the original base line was restored. The change in amplitude of Phe produced contraction in comparison to that elicited in

the control group was used to quantify changes in reactivity to Phe in different groups **(Cocks et al., 1988).** To investigate the relaxant effect of acetylcholine (Ach), pre-contraction with Phe was carried out first using a concentration that produces approximately 60–70% of the maximum contractile response. A cumulative concentration- response curve was made. This was obtained by doubling the Ach concentration without washing out the preparation, allowing enough time for each relaxation to produce its full effect. The tissue was gradually relaxed as the Ach concentration was increased, and the response was recorded on a slowly moving smoked drum. The concentration used was ranging from (10^{-7} - 10^{-4} M). The preparation was extensively washed after the last concentration of 10^{-4} M, until the original base line was restored. The amplitude of Ach-induced relaxation in comparison to that elicited in the control group was used to quantify changes in reactivity to Ach in different groups **(Cocks et al., 1988)**.

- **Isolated perfused heart experiment protocol (Langendorff model)**

Apparatus: Langendorff perfusion apparatus**.** ***Solution:*** The perfusate consisted of the modified Krebs-Henseleit buffer which was prepared daily with highly purified water with following composition (in mM): [NaCl (118.5), $NaHCO_3$ (25.0), d-glucose (11.0), KCl **(**4.7), $MgSO_4$ **(**1.2), KH_2PO_4 **(**1.2) and $CaCl_2$ (1.8) bubbled with 5% CO_2 and 95% O_2 **(Sutherland & Hearse, 2000).** ***Procedure:*** An intraperitoneal injection of pentobarbitone sodium solution at a final dose of 0.2-0.4g/Kg bodyweight was used to anaesthetize the rats and anticoagulant heparin sodium was co-administered at a dose of up to 5000 units per Kg bodyweight. The hearts were excised and quickly attached into the cannula of a gravity-driven Langendorff perfusion equipment upon the onset of profound anaesthesia, which was identified as the disappearance of the pedal pain withdrawal reflex, slowing of heart rate, and breathing **(Bell et al., 2011).** The heart is cannulated through the aorta and retrogradely perfused and suspended within a warmed organ chamber, allowing for a constant pressure perfusion system. The hearts temperature were kept at a constant temperature of 36-38^{o}C. Myocardial contractility was measured using a linear force contraction with a tie through the apex and connection to an isometric force transducer. A data acquisition hardware (PowerLab/8 SP by AD Instruments, Oxford, UK) was used to record the cardiac contractility and the results were displayed using the LabChart software (v7.3.8, AD Instruments, Oxford, UK) **(Bell et al., 2011).** ***Experimental design:*** *Ex vivo* rat hearts were equilibrated for about 40 min before starting the experiment. Data was obtained over a 20 minute baseline period, followed by 20 min exposure to five escalating concentrations of dapagliflozin. (0.001, 0.01, 0.1, 1.0, 10.0 µM). Absolute contractility data was adjusted to baseline levels by taking one-minute averages.

- **Statistical *analysis***

The obtained results were tabulated as means ± standard error (SE) of mean. One way analysis of variances (one-way ANOVA) was used for Comparison between different groups, followed by Post-Hoc (least significant difference "LSD") tests as described by **Armitage and Berry (1994)**. When p 0.05, the differences were considered significant. Statistical Package of Social Sciences (SPSS) computer software (version 21) was used to carry out the statistical analysis.

Results

Effect of dapagliflozin on HW, BW, HW/BW, blood pressure, HR, serum glucose, insulin and HbA1C values

DM.HF group showed insignificant change in HW, BW and HW/BW values compared with DM sham group. Dapagliflozin produced significant decrease in HW and HW/BW values compared with DM.HF group (Table 1).

DM.HF group showed insignificant change in SBP, DBP, MAP and HR values compared with DM sham group. Dapagliflozin produced significant decrease in SBP and insignificant change in DBP, MAP and HR values compared with DM.HF groups (Table 1).

DM.HF group showed significant increase in serum glucose and HbA1C levels compared with DM sham group. Dapagliflozin produced significant decrease in serum glucose and HbA1C levels compared with DM.HF groups (Table 1).

Table 1 Effect of dapagliflozin on HW, BW, HW/BW, blood pressure, HR, serum glucose, insulin and HbA1C values

Group n=6 / Parameter	CN	DM sham	DM.HF	DM.HF.dapa
HW (g)	1.32 ± 0.019	1.47 ± 0.029*	1.46 ± 0.024*	1.33±0.028#$
BW (g)	453±10.786	383.20± 10.590*	395.40±11.409*	426.20±8.794#
HW/BW (mg/g)	2.86±0.062	3.84±0.055*	3.70±0.098*	3.13±0.040*#$
SBP (mm Hg)	105.40 ± 1.470	147.00± 1.612*	142.00 ± 2.345*	130.00±2.429*#$
DBP (mm Hg)	81.20±2.311	98.20±1.562*	92.00±4.183*	88.80±2.871#
MAP (mm Hg)	88.80±1.772	114.40±0.678*	108.60±3.429*	102.40±2.421*#
HR (beats/min)	365.80±4.933	346.80±5.054	339.60±5.988*	342.40±11.170*
serum glucose (mg/dl)	94.98 ± 4.857	179.20 ± 5.860*	264.90 ± 14.090*#	152.20±9.372*$
Serum insulin (ng/ml)	3.31 ± 0.292	1.00± 0.016*	0.980±0.009*	0.964±0.016*
HbA_1C (%)	4.4±0.247	6.88±0.127*	7.76±0.155*#	6.35±0.242*$

Data represent means ± SE

HW, heart weight; BW, body weight; HW/BW, heart weight/ body weight; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; HbA1C, glycated hemoglobin A1C

* Significantly different from CN group

Significantly different from DM sham group

$ Significantly different from DM.HF group

Effect of dapagliflozin on serum levels of Cardiac-Specific enzymes and fibrotic markers.

DM.HF group showed significant increase in serum levels of CK-MB, LDH, cTnI, PICP, PIIINT and TGF-β1 compared with DM sham group. Dapagliflozin produced significant decrease in serum levels of CK-MB, LDH, cTnI, PICP, PIIINT and TGF-β1 compared with DM.HF groups (Table 2).

Table 2 Effect of dapagliflozin on serum levels of Cardiac-Specific enzymes and fibrotic markers.

Group n=6 / Parameter	CN	DM sham	DM.HF	DM.HF.dapa
CK-MB (u/ml)	125.14 ± 2.546	164.10 ± 5.866*	202.65 ± 6.118*#	145.12±6.385*#$
LDH (u/ml)	69.88±2.286	84.84± 3.177*	107.44±8.100*#	88.14±3.745*$
cTn1 (ng/ml)	0.34±0.049	0.716±0.065*	1.48±0.094*#	0.56±0.049*$
PICP (pg/ml)	24.32 ± 2.031	48.86 ± 3.123*	95.20 ± 2.107*#	65.60±3.203*#$
PIIINP (pg/ml)	2.62±0.455	9.78± 1.908*	26.60 ±3.203*#	15.38±1.88*$
TGF-β1 (pg/ml)	40.90±2.853	94.72± 4.502*	175.56±4.761*#	61.28±6.320*#$

Data represent means ± SE
CK-MB, Creatine Kinase-MB; LDH, Lactate Dehydrogenase; cTnI, Cardiac Troponin I; PICP, Procollagen I C-terminal Propeptide; PIIINT, procollagen III N-terminal peptide; TGFβ 1, transforming growth factor beta 1
* Significantly different from CN group
# Significantly different from DM sham group
$ Significantly different from DM.HF group

Effect of dapagliflozin on inflammatory mediators, anti-oxidative markers, and apoptotic mediators levels.

DM.HF group showed significant increase in serum levels of TNF-α, MDA and Caspase 3 and decrease in SOD and BCL2 level compared with DM sham group. Dapagliflozin produced significant decrease in serum levels of TNF-α, MDA and Caspase 3 and significant increase in SOD and BCL2 level compared with DM.HF groups (Table 3).

Table 3 Effect of dapagliflozin on inflammatory mediators, anti-oxidative markers, and apoptotic mediators levels.

Group n= 6 / Parameter	CN	DM sham	DM.HF	DM.HF.dapa
TNF-α (pg/ml)	16.16 ± 1.265	64.30± 3.648*	112.60± 4.966*#	44.55±3.203*#$
MDA (nmol/ml)	34.92 ± 2.817	71.36 ± 1.933*	156.72 ± 5.639*#	53.78±5.612*#$
SOD (u/ml)	26.02±1.454	17.12± 1.089*	10.88±1.253*#	18.92±1.196*$
BCL-2 (pg/ml)	233.24 ± 3.827	194.48 ± 3.788*	117.26 ± 9.555*#	193.50±5.342*$
Caspase 3 (ng/ml)	2.07±0.107	4.22± 0.220*	7.66±0.251*#	3.40±0.415*#$

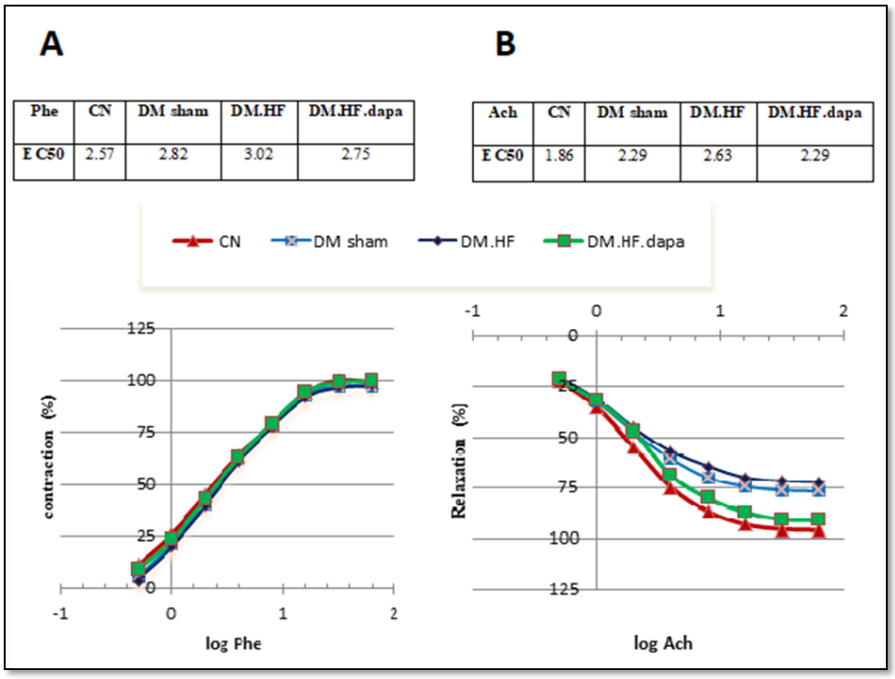
Data represent means ± SE
TNF-α, tumor necrosis factor-alpha; MDA, Malondialdehyde; SOD, super oxide dismutase
* Significantly different from CN group
# Significantly different from DM sham group
$ Significantly different from DM.HF group

I. Ex-vivo:

- ***Vascular reactivity***

The contractile activity of aortic strip in DM.HF.dapa group showed insignificant difference compared with DM.HF group. From graded dose response curve the half maximal effective concentration (EC50) values of vasomotor responses to Phe in CN, DM sham, DM.HF and DM.FH.dapa groups were 2.57, 2.82, 3.02, and 2.75 μM respectively (figure 1A).

The relaxation function of aortic strip in DM.HF group is markedly less compared to those of CN, while dapagliflozin treatment of this group induced significant preservation of these depressed responses. From graded dose response curve the EC50 values of relaxation responses to Ach in CN, DM sham, DM.HF and DM.HF.dapa groups were 1.86, 2.29, 2.63 and 2.29 μM respectively (figure 2B).



Phe	CN	DM sham	DM.HF	DM.HF.dapa
EC50	2.57	2.82	3.02	2.75

Ach	CN	DM sham	DM.HF	DM.HF.dapa
EC50	1.86	2.29	2.63	2.29

Figure (1): Effects of dapagliflozin treatment on contractile activity of aortic strip. (A) Concentration dependent contractile responses of aortic strips to phenylephrine [Phe (10−7–10−4 M)] stimulation in a manner of cumulative applications with EC50 values. (B) Concentration dependent relaxant responses of aortic strips to acetylcholine [Ach (10−7–10−4 M)] in a manner of cumulative applications on pre-contracted aortic strips with 16 µM Phe. The EC50 values from DM.HF.dapa comparison with those of CN, DM sham or DM.HF were given in tables as an inset. The maximum responses to Ach stimulation with high concentrations in DM.HF were markedly less compared to those of CN, while DAPA treatment of this group induced significant preservation of these depressed responses. The total number of rats for aortic strips/group; n=6.

- ***Effect of dapagliflozin on myocardial contractility.***

Ex vivo perfused hearts showed insignificant change on myocardial contractility and heart rate in response to escalating concentrations of dapagliflozin (0.001, 0.01, 0.1, 1.0, 10.0 µM).

II. Photomicrographs for histo-pathological findings:

Histo-pathological study of heart sections stained with H&E of DM.HF group showed loss of some cardiac muscles with other hypertrophied muscles and some inflammatory cells with deposition of collagen in the intermuscular spaces. While, DM.HF.dapa group showed attenuation of the hypertrophied myocytes with mild myocytes disarray (figure 2).

Histo-pathological study of liver sections stained with H&E of DM.HF group showed dilated congested sinusoids and marked portal inflammation with proliferation of bile ducts. While, DM.HF.dapa group showed nearly normal hepatic lobule with signs of regeneration seen in many binucleated hepatocytes and mild inflammation (figure 3).

Histo-pathological study of lung sections stained with H&E of DM.HF group showed marked inflammatory infiltrate and heart failure cells in the alveolar spaces and marked thickening of the alveolar wall with infiltration by inflammatory cells. While, DM. HF.dapa group showed mild thickening of the alveolar walls with mild inflammatory infiltrate (figure 4)

Histo-pathological study of heart sections stained with Masson trichrome of DM.HF group showed marked deposition of collagen fibers. While, DM.HF.dapa group showed minimal collagen fibers deposition in between cardiac muscles and the surrounding vessels (figure 5)

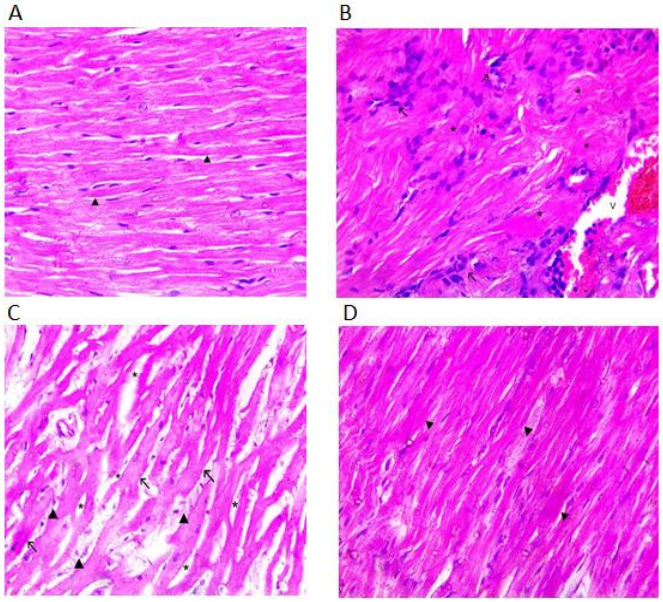


Figure 2: Photo micro-graph of myocardium **A.** CN group shows normal structure and thickness of cardiac muscles (arrowheads), **B.** DM sham group shows congested blood vessels (V) surrounded by wide areas of myocytes disarray (asterisks). Large numbers of fibroblasts (arrows) are located between muscle fibers, **C.** DM.HF group shows loss of some cardiac muscles (asterisks) with other hypertrophied muscles (arrows) and some inflammatory cells (arrowheads) with deposition of collagen in the intermuscular spaces and **D.** DM.HF.dapa group shows hypertrophied myocytes (arrowheads) with mild myocytes disarray **(H & E x 400).**

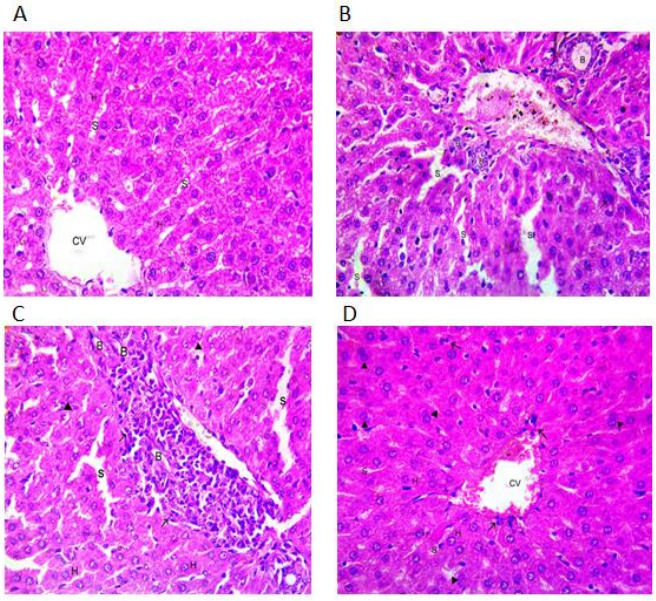


Figure 3: Photo micro-graph of liver tissue **A.** CN group shows normal architecture of liver lobules, **B.** DM sham shows markedly dilated sinusoids with regenerative hepatocytes (binucleation shown by arrows). The portal tract shows bile duct proliferation, **C.** DM.HF group shows dilated congested sinusoids and marked portal inflammation (arrows) with proliferation of bile ducts. Some hepatocytes are binucleated (arrowheads) and **D.** DM.HF.dapa group shows nearly normal hepatic lobule with signs of regeneration seen in many binucleated hepatocytes (arrowheads) and mild inflammation (arrows). CV, Central vein; H, Hepatocyte; S, sinusoid; B, Bile duct.

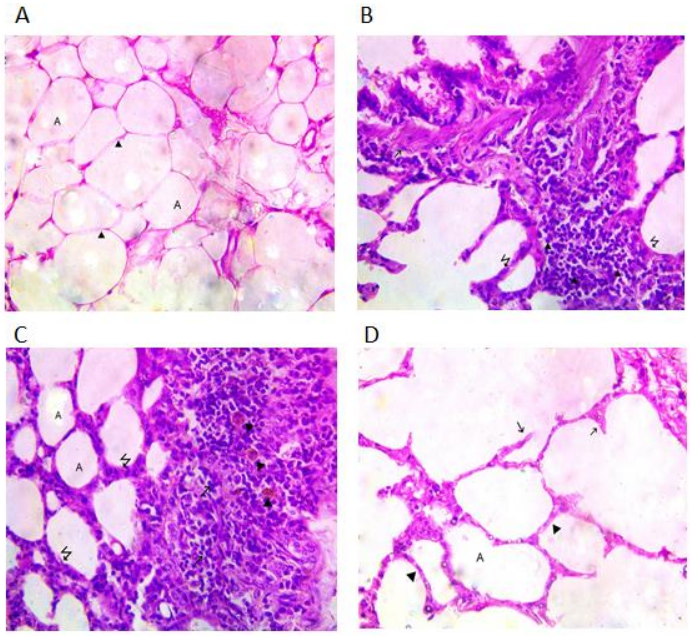


Figure 4: Photo micro-graph of lung tissue **A.** CN group shows normal alveolar walls (arrowheads), **B.** DM sham group shows thickening (broken arrows), infiltration by inflammatory cells (arrowheads) of the alveolar septa with destruction of some of them and marked interstitial inflammatory infiltrate could be seen with prominent smooth muscle hypertrophy (arrows) of the bronchial wall, **C.** DM.HF group shows marked inflammatory infiltrate and heart failure cells (arrowheads) in the alveolar spaces (right side) and marked thickening of the alveolar wall (broken arrows) with infiltration by inflammatory cells (arrows) and **D.** DM. HF.dapa group shows mild thickening of the alveolar walls (arrowheads) with mild inflammatory infiltrate. Some alveoli show destruction of the alveolar septa (arrows)**.** A, Alveoli

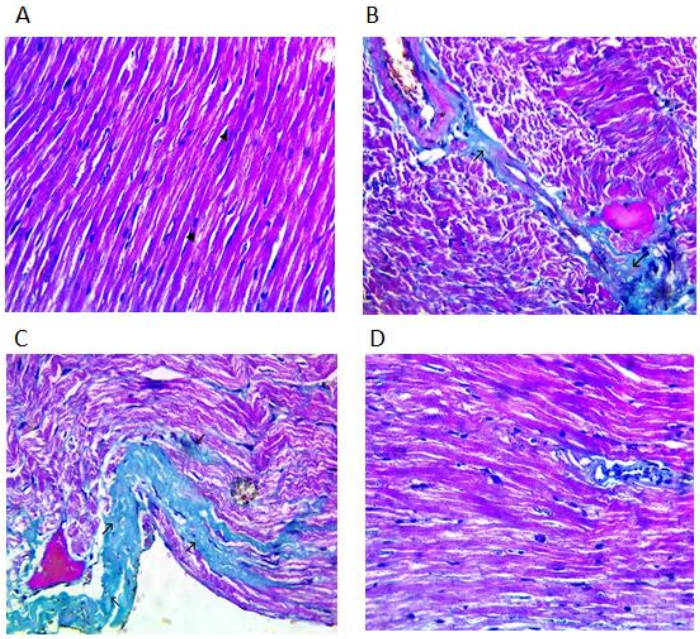


Figure 5: Photo micro-graph of myocardium **A.** CN group shows normal cardiac muscles (arrowheads) without collagen deposition, **B.** DM sham group shows deposition of collagen fibers (arrows) surrounding thickened blood vessels (asterisk), **C.** DM.HF group shows marked deposition of collagen fibers (arrows) and **D.** DM.HF.dapa group shows minimal collagen fibers deposition in between cardiac muscles and the surrounding vessels **(Masson trichrome x400).**

Discussion

Cardiac remodeling after MI is a complex process that seriously threatens the patient's life. Its pathophysiological mechanisms include fibrosis, inflammation, apoptosis, and other pathological processes. These pathological factors contribute to the development of HF after MI, which indicate that it's important to minimize their negative effects as soon as possible **(Bahit et al., 2018)**

Recent studies have shown that in addition of improving hyperglycemia, the SGLT2 inhibitor dapagliflozin also has a positive effect on patients with HF **(Petrie et al., 2020; Kosiborod et al., 2020)**. It has been approved by the FDA in 2020 for the treatment of adult patients with HFrEF (with or without T2D) to decrease the danger of cardiovascular death and hospitalization for HF. But, how SGLT2 inhibitors improve prognosis in HFrEF remains unknown **(Murphy et al., 2020)**. There is still a controversy on the Dapagliflozin's efficacy and clinical application, and there are no standardized clinical guidelines for reference (**Cai et al., 2021)**.

The results of the present study showed that dapagliflozin has a beneficial effect in HF induced diabetic rats. Administration of dapagliflozin improved hyperglycemia and cardiac hypertrophy as well as decreased the serum level of CK-MB, LDH, cTn1, PICP, PIIINP, TGF-β1, TNF-α and MDA, and cardiac level of Caspase 3, while increased the serum levels of SOD and BCL2. Besides that, administration of dapagliflozin improved the histopathological picture of the heart, liver and lung and improved the vascular reactivity of the aorta.

The result of the present study showed that DM.HF group of rats was associated with no difference in HW and HW/BW compared with DM sham group. These results are consistent with that obtained by **Solomon et al., (2002)** who found that diabetes is associated with HF at lower ventricular volumes and stated that cardiac remodeling in diabetic individuals differs from that in non-diabetic subjects, with lesser degrees of ventricular dilatation demonstrated following MI. This may be partially explained by various morphological and functional myocardial alterations associated with diabetes. These include increased collagen content, myocardial fibrosis, and hypertrophy which may explain the tendency of diabetics to develop diastolic dysfunction and smaller ventricular cavities more than non-diabetic subjects **(Liu et al., 2001).**

The results of the present work demonstrated that therapeutic administration of dapagliflozin decreased HW, HW/BW and increased BW of HF induced -diabetic rats. These results were in accordance with **Tian et al. (2021)** who founded that the significantly elevated HW to BW ratio observed in T2D cardiomyopathy hearts, showed reduction after dapagliflozin treatment. Also **Chang et al. (2021)** studied the protective effect of oral dapaglifozin on doxorubicin -induced cardiotoxicity in diabetic rats. They founded that doxorubicin -induced cardiac toxicities were observed through an increase in the ratio of heart to body weight in the doxorubicin -treated STZ rats while dapaglifozin signifcantly alleviated injuries.

The present finding showed that DM.HF group of rats was associated with no difference in SBP, DBP, MAP and HR compared with DM sham group. These results are in accordance with **Malfitano et al. (2014)** who investigated the association between hyperglycemia and myocardial infarction on cardiovascular autonomic modulation in rats 30 days after buffer or STZ injection, and 15 days after MI. They found that SBP

remained the same for all groups (Control, MI, and diabetic MI) and DBP was decreased after diabetes and MI when compared to controls. The decrease in DBP in diabetic MI group may be related to cardiovascular autonomic neuropathy **(Rodrigues et al., 2013).**

The results of the present study showed that therapeutic administration of dapagliflozin not alters DBP, MAP and HR of DM.HF group of rats. While, caused reduction in their SBP. These result was in parallel with **Arow et al. (2020)** who investigated the effect of dapagliflozin, on diabetic cardiomyopathy in diabetic mice and reported that concomitant oral administration of dapagliflozin (1.5 mg/kg/day) reduced blood pressure. In addition, **Shi et al. (2019)** reported that administration of dapagliflozin by gavage (1.0 mg/kg/day) for 4 weeks in the transverse aortic constriction (TAC) induced -cardiac remodeling mice model caused no difference in HR and SBP that may affect cardiovascular outcomes. The reason for which fluid deplete seldom induce RAAS and HR alterations should be attributed to the sympathetic inhibition by SGLT2 inhibitor with unidentified pathway **(Elliott et al., 2016)**

The present finding showed that rats of DM.HF group was associated with significant increase in fasting serum glucose and Hb A1C levels compared with DM sham group. The results of the present work were in accordance with **Guglin et al. (2014)** whose study was to explore longitudinal changes in glycosylated hemoglobin (HbA1C) in patients with diabetes with advanced HF which concludes that left ventricular assist devices improve blood glucose control in diabetes patients. They hypothesized that the decreased physical activity in HF patients may lead to decreased insulin sensitivity and to compensatory increased insulin requirements and hyperglycaemia. In addition, increased catecholamine's levels and sympathetic activity stimulate gluconeogenesis and glycogenolysis. Furthermore they hypothesized that the haemodynamic consequences accompanying HF (decreased forward blood flow and increased central venous pressure) lead to hypoperfusion and congestion of the pancreas and liver, which may impair their ability to regulate metabolic homeostasis.

These results also were in agreement with **Andersson et al. (2010)** who made a nationwide cohort study and stated that HF severity predicts the risk of developing diabetes after MI. They explained that, HF is a state of high insulin resistance. In T2D, patients usually have a combination of insulin resistance and insulin deficiency. Patients with HF have insulin sensitivity which is 58% lower than that in healthy subjects (**Wong et al., 2008)**. High levels of catecholamines which are typically present in HF may contribute to this condition. Increased sympathetic activity inhibits pancreatic insulin secretion and stimulates hepatic gluconeogenesis and glycogenolysis, leading to hyperglycemia and therefore increasing insulin requirements **(Heck and Dutka, 2009)**. Another consideration is that pancreas, similar to liver and kidneys, is exposed to two major hemodynamic abnormalities characteristic for chronic HF such as decreased forward blood flow (low output) and increased central venous pressure (congestion) **(Guglin et al., 2014)**.

The results of the present work demonstrated that therapeutic administration of dapagliflozin significantly decreased fasting serum glucose and Hb A1C levels in DM.HF group of rats compared with DM sham group. These results were in accordance with **Chang et al. (2021)** who studied the protective effect of dapagliflozin on

doxorubicin-induced cardiotoxicity in diabetic rats. Pretreatment with dapaglifozin decreased blood glucose compared with the doxorubicin -treated diabetic rats.

The present finding showed that DM.HF group of rats showed significant increase in cardiac-Specific enzymes CK-MB, LDH and TnI, levels compared with DM sham group. These results are in accordance to **Peng et al. (2021)** who found that CK-MB level significantly increased in isoproterenol-induced HF rats compared with control group. Also **Li et al. (2021)** stated that 4 weeks after coronary artery ligation, the concentrations of CK-MB, were significantly higher in the MI group compared to the sham group**.**

The results of the present work are in accordance with **Ueda et al. (2020)** who revealed that the serum cTnI concentration was significantly higher in animals with echocardiographically apparent cardiac disease as compared with the control group. In addition **Haroon et al. (2021)** reported a significant increased serum level of LDH, and presence of cTnI in rats with doxorubicin -induced cardiotoxicity in comparison with normal control. Also **Xie, & Li, (2020)** observed increase of CK-MB and LDH activity in acute heart failure (AHF) rats four weeks after induction of AHF.

The results of the present work showed that therapeutic administration of dapagliflozin in HF -induced diabetic rats significantly decreased cardiac-Specific enzymes CK-MB, LDH and TnI levels compared with DM sham group. These results are in agreement with **Gong et al. (2020),** who used dapagliflozin as a preventive measure, reported that pretreated rats with dapagliflozin showed significant reduction in the levels of CK-MB, and hyper-tensive cardiac troponin I (hs-cTNI) compared with the ischemia/reperfusion group. And stated that this cardioprotective effects of dapagliflozin were confirmed through activating the PI3K/AKt/mTOR signaling pathway.

Cardiac fibrosis following MI is induced by fibroblasts proliferation and exaggerated accumulation of collagen type I and type III in interstitial and perivascular areas. It is a maladaptive process that leads to left ventricular (LV) remodeling and dysfunction, and poor outcomes **(González et al., 2018)**. In this regard, increased collagen cross-linking is associated with LV stiffness and diastolic dysfunction in HF. The realignment of collagen and cardiomyocytes impair transmission of cardiomyocyte force and myocardial contractility. Deposition of fibrotic tissue increases oxygen diffusion distance leading to the impairment of oxygen supply to the cardiomyocytes (**Villari et al., 1993)**.

PICP and PIIINP are circulating biomarkers of myocardial interstitial fibrosis (MIF) **(González et al., 2018)**. Serum PICP concentration correlates with collagen type I volume fraction (CIVF) in HF. Serum PICP is generated during the extracellular conversion of procollagen type I into collagen type I by the enzyme bone morphogenetic protein-1 or procollagen carboxy-terminal proteinase (**Prockop and Kivirikko, 1995**). A net release from the heart into the circulation has been reported in HF, suggesting a cardiac origin for systemic PICP in this syndrome (**Querejeta et al., 2004**).

Serum PIIINP concentration correlates with collagen type III volume fraction (CIIIVF) in HF **(Klappacher et al., 1995)**. Most serum PIIINP is generated during the extracellular conversion of procollagen type III to collagen type III by the enzyme procollagen aminoterminal proteinase **(Prockop and Kivirikko, 1995)**. Serum

PIIINP levels correlated with the severity and outcomes in HF of different causes regardless of ejection fraction **(Krum et al., 2011; Lopez et al., 2015)**

In our study, DM.HF group of rats showed significant increase in PICP and PIIINT serum levels and therapeutic administration of dapagliflozin lowered their serum level. The results of the present work are in accordance to **Lo´pez et al. (2015)** who stated that the PICP is associated with raised collagen content on endomyocardial biopsy, the gold standard measure of cardiac fibrosis **(Whittaker et al., 1994)**, diastolic dysfunction and prognosis in HF with preserved ejection fraction (HFpEF). Also **Lombardi et al. (2003)** stated that the PIIINP has been detected in serum and are elevated in hypertrophic cardiomyopathy (HCM) patients. While the result of the present work was in contrary to **Adamcova et al. (2019)** who found that there was no correlations between plasma markers of collagen (PICP and PIIINP) and collagen content or molecular markers of collagen in the LV in isoproterenol induced heart damage model. They explained their results that, serum procollagens mirror the rate of collagen deposition, not the amount of collagen deposition. In their study, although collagen content in the LV was increased, PICP and PIIINP levels were significantly reduced in the isoproterenol group and no correlation between plasma markers of collagen and collagen content were found. However, both PICP and PIIINP correlated with BW, which was reduced by 25% in the isoproterenol group. The cardiac cachexia observed in individuals with heart failure seems to be the result of prolonged hypoperfusion of gastrointestinal tract and skeletal muscle, concomitant anaemia, renal dysfunction and cytokines production and reflects a catabolic state (**von Haehling et al., 2013; Ishida et al., 2017**). The reduction of BW in the isoproterenol group presumably associated with the catabolic condition during HF might result in slowing-down the rate of left ventricular collagen turnover and even in the plasma proteins degradation, thus reducing the plasma level of myocardial fibrosis markers. Their explanation was in accordance to **Jensen (1997)**, who described the kinetics of PIIINP under different pathophysiological conditions. Under the given steady state conditions, the turnover of PIIINP was well reflected by changes in plasma PIIINP, but this relation disappeared when the body was in a catabolic state. Conditions with increased anabolism gave rise to increased serum concentrations of PIIINP as compared with normal states.

TGF-β1 has been identified as a key regulator of cardiac fibrosis, which has wide-ranging effects, including increase collagen and matrix protein production, maintain fibroblast viability, and inhibit production of metalloproteinase **(Lijnen et al., 2000; Biernacka et al., 2011).** By binding with its receptor in the plasma membrane, TGF-β1 induces phosphorylation of Smad2/3 transcription factor which mediate canonical signaling. Phosphorylated Smad2/3 combines with Smad4 in the cytoplasm and translocate to the nucleus to induce transcriptions of fibrosis related genes, including collagen I and collagen III **(ten Dijke & Arthur, 2007).**

The result of the present work showed that DM.HF group of rats showed significant increase in TGF-β1 serum level and therapeutic administration dapagliflozin lowered their serum level. The results of the present work are in accordance to **Chen et al. (2019)** who stated that the expression level of TGF-*β*1 mRNA was significantly increased in the MI rat model group after 4 weeks of LAD coronary artery ligation compared with the sham group. Also **Yang et al. (2021)** stated that permanent LAD-

ligation resulted in a significant up-regulation of TGF-β1 expression levels in rat myocardial tissue 28 days after ligation compared with sham group. In the same consistence **Tian et al. (2021)** reported that the transcription level of fibrotic markers including TGF-β were observed in the untreated diabetic cardiomyopathy group and then reduced after dapagliflozin treatment. They explained that dapagliflozin exerts its antifibrotic effect by blocking the fibroblast origin and directly suppressing the activation of cardiac fibroblasts (CFs). Dapagliflozin alleviates cardiac fibrosis through suppression of endothelial-to-mesenchymal transition and fibroblast activation via AMPKα-mediated inhibition of TGF-β/Smad signalling. AMP-activated protein kinase (AMPK) is a member of the serine/threonine (Ser/Thr) kinase family, which acts as a 'fuel gauge' under cell stress conditions to maintain energy balance, using an α catalytic subunit as its principal functional domain **(Feng et al., 2018)**. Reduced AMPKα activity has been observed in failing human and animal hearts and is closely related to cardiac fibrosis **(Qi et al., 2017; Li et al., 2019).** Moreover, based on the close connection between Smad4 and cardiac fibroblast (cFs) activation (**Huang et al., 2014)**, inhibited Smad4 expression may account for the direct suppression of CFs activation by dapagliflozin. Also, **Lee et al., (2017)** stated that SGLT2 inhibitors have an inhibitory effect on cardiac fibrosis and explained that by augmenting the activation of M2 macrophages and restraining myofibroblast differentiation, thus showing significant antifibrosis effect.

Cardiomyocytes apoptosis was also involved in the pathological process of the left ventricular remodeling after MI **(Danial and Korsmeyer, 2004).** Abnormal changes or damages of mitochondria after MI were involved in apoptosis of cardiomyocytes in the infarcted area **(Frangogiannis, 2015).** Cytochrome C and other apoptosis-promoting active proteins located in the gap between mitochondrial membranes are released into the cytoplasm, which mediated recruitment of pro-caspase 9 and eventually leaded to the activation of caspase 3 **(Allan and Clarke, 2009; Frangogiannis, 2015).** Since caspase 3 was considered important participants to initiate apoptosis signaling pathway, the apoptosis markers (caspase 3 and Bcl-2) were detected.

The result of the present work showed that DM.HF group of rats showed significant increase in caspase 3 level in cardiac tissue and decrease in Bcl2 serum level. Therapeutic administration dapagliflozin lowered caspase 3 level and caused increase in Bcl-2 serum level. These results are in accordance to **Chen et al. (2019)** who stated that levels of caspase3 were significantly increased in the MI rat model group after 4 weeks of left anterior descending coronary artery ligation compared with the sham group. Also **Ren et al. (2020)** results showed that, 4 weeks after MI in rats through LAD coronary artery ligation, Bcl-2 protein expression in heart tissues was significantly decreased in the MI group when compared with sham group. In the same consistence **Wu et al. (2021)** observed a significant increase in myocardial apoptosis rate in the HF group and with the occurrence of HF the expression of the antiapoptotic factor Bcl-2 was suppressed.

The results of the present work are in agreement with **Lin et al. (2021)** who investigated thoroughly a potential protective role of dapagliflozin administration in a mitral regurge -induced HF rat model. They measured the apoptosis-related proteins, cleaved caspase 3, and Bcl-2, in cardiac tissue by Western blot. Compared with Sham rats, cleaved caspase 3 was markedly upregulated in LV region of MR rats in which MR

induced left heart dilatation and functional decline, while being significantly suppressed in LV of rats treated with dapagliflozin. Conversely, the expression of antiapoptotic protein Bcl-2 was significantly downregulated in LV region of MR rats but preserved in MR rats treated with dapagliflozin. Also **Chang et al. (2021)** studied the protective effect of dapagliflozin on doxorubicin -induced cardiotoxicity in diabetic rats. The apoptosis-related proteins, including cleaved caspase 3, and Bcl-2, were measured in the cardiac tissue. Their results showed that doxorubicin treatment markedly upregulated the levels of pro-apoptotic protein cleaved caspase 3. Conversely, Bcl-2 expression, a key regulator of apoptosis, was markedly downregulated in the doxorubicin-treated STZ rats, whereas pretreatment with dapaglifozin preserved the expression of Bcl-2. **Gong et al. (2020)** explained the reduction of apoptosis mediated by dapagliflozin by the activation of the PI3K/AKt/mTOR signaling pathway **(Maiese et al., 2012).**

Reactive oxygen species (ROS) are involved in many signaling pathways**.** The overproduction of ROS serves a significant role in organ damage associated with various diseases, including heart injury **(Kura et al., 2020).** In the present study, the results showed increase in MDA and decrease in SOD serum levels in DM.HF group of rats. Therapeutic administration of dapagliflozin lowered MDA level and caused increase in SOD serum level. These results are in accordance to **Chen et al. (2021)** who stated that oxidative stress was enhanced in the hearts of HF rats. SOD activity level was reduced in the LVs of MI-induced HF rats. MDA, superoxide anions activity levels were significantly increased in LV of MI-induced HF rats compared with sham operated group. Also **Yang et al. (2021)** stated that LAD-ligation resulted in a significant decrease in SOD level and a significant increase in MDA level after 28 days of ligation compared with Sham group.

Arow et al. (2020) suggest that dapagliflozin reduced intracellular calcium overload thereby reducing ROS production in the diabetic model of cardiomyopathy. Protecting the mitochondria, attenuating inflammation and preventing fibrosis would be consecutive to reducing ROS. While **Tian et al. (2021)** stated that, dapagliflozin may be capable of alleviating DCM through the AMPKα-mediated inhibition of oxidative stress, generated by NADPH oxidases.

The increase in the pro-inflammatory cytokine profile is a responsive tissue repair mechanism (**Schumacher& Naga Prasad, 2018)**. TNFα is among the most common pro-inflammatory cytokines that directly contribute to myocardial remodeling and dysfunction after MI, progressively leading to HF **(Bartekova et al., 2018). Berthonneche et al., 2004** explained that TNF-α has been shown to modulate cardiovascular function by a variety of mechanisms. It caused depression in myocardial contractility by uncoupling β-adrenergic signaling**,** increasing cardiac nitric oxide and peroxynitrite**,** or altering intracellular calcium homeostasis**.** TNF-α also induce structural changes in the failing myocardium, such as interstitial fibrosis**,** cardiomyocyte hypertrophy**,** and dilation**.** Besides, TNF-α has been shown to promote cardiomyocyte apoptosis**.** It may also activate metalloproteinases and impair the expression of their inhibitors.

The result of the present work showed significant increase in TNF-α serum level in DM.HF group of rats and therapeutic administration of dapagliflozin caused significant decrease in its level. These results were in agreement with **Hu et al. (2021)** who stated that the levels of the inflammatory cytokines including TNF-α were significantly higher

in HF rats at 12 weeks after left anterior descending coronary artery ligation compared to control rats. Also **Yang et al. (2021)** Stated that permanent LAD-ligation led to a significant increase in serum TNF-α in rats after 28 days of ligation compared with Sham group.

In the same consistence , the results of the present work is in agreement with **ye et al., (2017)** who founded that myocardial mRNA levels of TNFα significantly increased in mice with T2D and these increases were attenuated by dapagliflozin 1 mg/kg/day administeration for 8 weeks.

Conclusion

Dapagliflozin is a potential drug for the treatment and improvement of HF, which showed a significant therapeutic effect in diabetic rats with HF caused by permanent LAD-ligation. The effect of dapagliflozin on ameliorating HF model is related to its ability to improve hyperglycemia, myocardial fibrosis, myocardial inflammation, inhibit oxidative stress, and cardiomyocytes apoptosis, besides improving vascular reactivity.

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