Dapagliflozin Guards Against Cadmium-Induced Cardiotoxicity via Modulation of IL6/STAT3 and TLR2/TNFα Signaling Pathways

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Received: 23 May 2022 / Accepted: 6 October 2022 / Published online: 15 October 2022
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Abstract
Cadmium (Cd) is a common environmental pollutant that leads to severe cardiotoxic hazards. Several studies were carried out to protect the myocardium against Cd-induced cardiotoxicity. Up till now, no research has evaluated the protective effect of dapagliflozin (DAP) against Cd induced cardiotoxicity. Thus, we aimed to explore the role of DAP in such model with deep studying of the involved mechanisms. 40 male Wistar albino rats were included in current study. Cd (5 mg/kg/day) was administered orally for 7 days to induce cardiotoxicity with or without co-administration of DAP in three different doses (2.5, 5, 10 mg/kg/day) orally for 7 days. Our data revealed that Cd could induce cardiotoxicity with significant increase in serum cardiac enzymes, heart weight, tissue malondialdehyde (MDA), tumor necrosis factor alpha (TNFα), nuclear factor kappa B (NFκB), toll like receptor2 (TLR2), interleukin 6 (IL6) and caspase3 immunoexpression with abnormal histopathological changes. In addition, Cd significantly decreased the level of heme oxygenase1 (HO1), nuclear factor erythroid 2-related factor 2 (Nrf2), signal transducer and activator of transcription (STAT3), reduced glutathione (GSH), glutathione peroxidase (GPx), and total antioxidant capacity (TAC). Co-administration of DAP could ameliorate Cd cardiotoxicity with significant improvement of the biochemical and histopathological changes. We found that DAP had protective properties against Cd induced cardiotoxicity and this may be due to its anti-oxidant, anti-inflammatory, anti-apoptotic properties and modulation of IL6/STAT3 and TLR2/TNFα-signaling pathway.

Keywords Dapagliflozin · Cadmium · Cardiotoxicity · Toll-like receptor · Interleukin6 · Signal transducer and activator of transcription

Introduction
Cadmium (Cd) is one of the most common toxic heavy metals that have severe cardiotoxic hazards especially on excessive exposure due to cigarette smoking or food and water contamination. Cd has a great ability to accumulate in our bodies for very long period [1]. Several mechanisms mediate Cd cardiotoxicity such as induction of oxidative stress with release of free radicals, metabolic disturbance and dyslipidemia. Cardiotoxicity of Cd leads to atherosclerosis, coronary spasm, ischemic injury, myocardial infarction, hypertension, and stroke [1, 2]. Uncontrolled and excessive formation of free radicals is the main cause that stimulates oxidative stress and disturbs cellular function leading to membrane lipid peroxidation, DNA damage, and cell death. Furthermore, heart cells are highly sensitive to the released reactive oxygen species (ROS) and the anti-oxidative system of these cells is fully saturated by the endogenous oxidative metabolic products [1].

Up-regulation of the different inflammatory pathways is considered as another important cascade in mediating Cd cardiotoxicity. Stimulation of pro-inflammatory cytokines including tumor necrosis factor alpha (TNFα), interleukin6 (IL6) and nuclear factor kappa B (NFκB) is accompanied with enhancement of Janus kinase/signal transducer and...
activator of transcription (JAK/STAT) which is a critical pathway during cardiac injury [3–5]. STATs are considered as a family of protein that could regulate gene expression of angiogenesis, inflammation and apoptosis. This family is pre-stimulated with IL-6 and mediates different cardiac disorders as hypertrophy, myocardial infarction and heart failure [6–8]. Besides that, toll-like receptors (TLRs) control innate immunity and inflammation inside the cell and increase the gene expression of different pro-inflammatory cytokines such as TNFα and interleukins that have a critical role in myocardial tissue injuries. We can conclude that modulation of the TLR2/TNFα and IL6/STAT3 pathways represents essential targets in controlling cardiac damage [9–13].

Sodium glucose co-transporter 2 (SGLT2) inhibitors including dapagliflozin (DAP) are the most recent oral anti-diabetic drug group. Its action is based on decreasing blood glucose level and inhibiting renal glucose reabsorption. Furthermore, DAP has non-glycemic effects including lowering of blood pressure, ability to control metabolic disorders and decreasing body weight [14–17]. DAP could decrease inflammatory cytokines such as TNFα, IL-1β, and IL-6 but increase anti-inflammatory one; IL-10, increase the M2 (anti-inflammatory)/M1 (pro-inflammatory) phenotypemacrophage ratio. In addition, DAP acts as an antioxidant and mediates M2 macrophage polarization through regulating STAT3 pathway with scavenging of reactive oxygen and nitrogen species followed by decrease of cardiac inflammation. Moreover, it controls the renin-angiotensin aldosterone system, decreases cardiac and renal complications of diabetes, improves cardiac ischemia–reperfusion injury and doxorubicin cardiotoxicity depending on its pharmacological properties but till now there are no data regarding its role in Cd cardiotoxicity [18–22].

Increasing the critical need for finding new cardioprotective agents against Cd cardiotoxicity and the recently detected cardiopreserving properties of DAP guided us to evaluate its ability to control Cd cardiotoxicity and to investigate the different mechanisms mediating such effect including TLR2/TNFα and IL6/STAT3 pathways.

Materials and Methods

Chemicals

DAP and Cd were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Caspase3 (bsm-33199M) monoclonal antibody was from Bioss Antibodies Inc, USA. Moreover, ELISA kits of TLR-2 (Catalog # MBS264416), IL6 (Catalog # MBS269892), cardiac enzymes of creatine kinase-MB (CK-MB) (Catalog # MBS2515061), Lactate dehydrogenase (LDH) (Catalog # MBS043166), heme oxygenase 1 (HO1) (Catalog # MBS764989), nuclear factor erythroid 2-related factor 2 (Nrf2) (Catalog # MBS1602926), troponin I (Catalog # MBS722833) were from My BioSource Co., San Diego, CA, USA. Total antioxidant capacity (TAC) (Catalog # TA2513) was obtained from Biodiagnostic, Egypt.

Animals and Experimental Design

40 male albino rats of Wistar species weighing about 200–250 g were included in current study. These animals were from Animal Research Centre, Giza, Egypt. Rats were kept in suitable conditions (3 rats/cage) and the duration of acclimatization was about 2 weeks with free access to chow and tap water. Our study was performed in accordance with the ethical standards of faculty of medicine, Minia University, Egypt in agreement with EU Directive 2010/63/EU for animal experiments.

Rats were randomly divided into 5 groups (n = 8 each).

Group I received the vehicle orally for 7 days.

Group II was given Cd (5 mg/kg/day) orally [23] for 7 days.

Group III was administered DAP (2.5 mg/kg/day) orally and Cd (5 mg/kg/day) orally [23] for 7 days.

Group IV was given DAP (5 mg/kg/day) [24] and Cd (5 mg/kg/day) orally [23] for 7 days.

Group V received DAP (10 mg/kg/day) [25] and Cd (5 mg/kg/day) orally [23] for 7 days.

Preparation of Samples and Storage

Rats were euthanized on 7th day and each rat was anesthetized by i.p. injection of 20% Urethane hydrochloride (1 mg/kg). Arterial blood of each rat was collected from abdominal aorta then centrifuged at 5000 rpm for 15 min to obtain the sera (JanetzkiT30 centrifuge, Germany) for measuring cardiac enzymes and TAC. Each heart was excised, washed with saline and weighed. One part of each ventricle was fixed in 10% formalin and embedded in paraffin for further histopathological and immunohistochemical examination and another part was kept at −80 °C. For preparing cardiac tissue homogenates specimens were homogenized in PBS 20%w/v using GLAS-Col homogenizer, USA then centrifuged at 3000 rpm for 20 min and the supernatant was separated and kept at −80 °C till used.

Biochemical Measurements

Assessment of Cardiac Enzymes (Troponin I, CK-MB and LDH) in Serum

Serum cardiac enzymes were measured using the available commercial ELISA kits according to the manufacturers’
instruction based on sandwich ELISA immunoassay technique. The microtiter plate was pre-coated with a monoclonal antibody specific for each parameter then standards or samples were added to the microtiter plate wells and the detected enzyme bound to the antibody pre-coated wells. The enzyme–substrate reaction was terminated by addition of a sulphuric acid solution and the color change was measured spectrophotometrically. The color intensity was proportional to the concentration of the detected enzyme.

**Evaluation of Oxidative Stress Parameters**

We used colorimetric kit for measuring TAC and results were expressed as mmol/ml. MDA was the major product of lipoperoxidation that was detected colorimetrically with the formation of MDA-thiobarbituric acid pink colored Schiff base adduct at 535 nm using 1, 1, 3, 3-tetramethoxypropane as standard and results were expressed as nmol/g tissue [26]. Measurement of GSH was based on binding of sulfhydryl group with Ellman’s reagent followed by formation of a yellow color that was detected colorimetrically at 405 nm by Beckman DU-64 UV/VIS spectrophotometer, USA as µmol/g tissue [27].

**Measurement of TLR2, HO1, Nrf2 and IL6**

Detection of these markers was performed according to manufacturer’s instructions of the available ELISA kit depending on the double-sandwich ELISA technique. The pre-coated antibody of the measured parameters and the detecting antibody were labeled with biotin. Samples and biotin labeling antibody were added into ELISA plate wells and washed out then Avidin-peroxidase conjugates were added to ELISA wells. Finally, yellow color formed and the intensity of the color was positively correlated with the testing factors in samples.

**Western Blotting of TNFα, NFκB, GPx and STAT3**

Heart tissue homogenate about 50 μg of total proteins were boiled for 5 min with a buffer containing 2-mercaptoethanol then loaded on 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with running for 2 h at 100 V. After electrophoresis, proteins were blotted to polyvinylidene fluoride (PVDF) membranes. Blocking step was for 1 h in a trisbuffered saline (TBS-T) and a blocking solution which contained 5% (w/v) solution.

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**Table 1** Effect of DAP on cardiac enzymes

| Groups   | CK-MB (U/L) | LDH (U/L) | Troponin I (U/L) |
|----------|-------------|-----------|------------------|
| CON      | 21.0 ± 1.0  | 143.8 ± 1.7| 15.0 ± 1.4      |
| Cd       | 89.4 ± 1.9ac| 274.4 ± 8.6ac| 84.4 ± 4.2ac    |
| Cd+DAP-LD| 78.0 ± 4.0abc| 237.0 ± 11.7| 65.4 ± 4.2abc  |
| Cd+DAP-MD| 51.3 ± 1.8abc| 193.9 ± 6.01abc| 3.01abc     |
| Cd+DAP-HD| 28.4 ± 0.8b | 175.5 ± 2.6 | 23.5 ± 1.5b    |

Values are representation of 8 observations as Mean ± SEM. Results are considered significantly different when P < 0.05

a Significant difference compared to control
b Significant difference compared to cadmium given group
c Significant difference compared to dapagliflozin high dose (10 mg/kg/day) plus cadmium given group

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**Table 2** Effect of DAP on oxidative stress parameters (MDA, GSH, TAC, HO1, Nrf2)

| Groups   | MDA (nmol/g tissue) | GSH (µmol/g tissue) | HO1 (ng/ml) | Nrf2 (ng/ml) | TAC (mmol/ml) |
|----------|---------------------|---------------------|-------------|--------------|---------------|
| CON      | 24.9 ± 1.2          | 939.7 ± 23.0        | 21.5 ± 1.4  | 55.5 ± 1.5   | 0.9 ± 0.05    |
| Cd       | 71.4 ± 2.8ac        | 305.6 ± 9.7ac       | 7.0 ± 0.7ac | 22.1 ± 1.5ac | 0.4 ± 0.02ac  |
| Cd+DAP-LD| 51.3 ± 2.6abc        | 394.9 ± 16.5abc     | 10.4 ± 1.1ac| 27.4 ± 1.7ac | 0.6 ± 0.02abc |
| Cd+DAP-MD| 40.4 ± 2.4abc        | 386.6 ± 18.3abc     | 12.6 ± 0.9abc| 40.6 ± 1.7abc| 0.7 ± 0.04abc |
| Cd+DAP-HD| 28.02 ± 1.8b         | 558.9 ± 6.3b        | 19.1 ± 0.7b | 51.4 ± 1.2b  | 0.8 ± 0.06b   |

Values are representation of 8 observations as Mean ± SEM. Results are considered significantly different when P < 0.05

a Significant difference compared to control
b Significant difference compared to cadmium given group
c Significant difference compared to dapagliflozin high dose (10 mg/kg/day) plus cadmium given group

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**Table 3** Effect of DAP on heart weight changes, TLR2, IL6

| Groups   | Heart weights (mg) | TLR2 (ng/ml) | IL6 (pg/ml) |
|----------|---------------------|--------------|-------------|
| CON      | 374.4 ± 25.2        | 4.0 ± 0.2    | 15.0 ± 1.4  |
| Cd       | 688.4 ± 25.4ac      | 13.8 ± 0.9ac | 84.4 ± 4.2ac|
| Cd+DAP-LD| 558.8 ± 43.5abc     | 10.75 ± 0.9abc| 65.4 ± 4.2abc|
| Cd+DAP-MD| 538.0 ± 22.3abc     | 8.1 ± 0.7abc | 53.5 ± 3.0abc|
| Cd+DAP-HD| 414.4 ± 26.0b       | 5.8 ± 0.4b   | 23.5 ± 1.5b |

Values are representation of 8 observations as Mean ± SEM. Results are considered significantly different when P < 0.05

a Significant difference compared to control
b Significant difference compared to cadmium given group
c Significant difference compared to dapagliflozin high dose (10 mg/kg/day) plus cadmium given group
non-fat milk and 0.05% Tween-20. Overnight incubation was at 4 °C with primary antibodies (1:1000) for rabbit anti-TNF-α (Catalog# 11948), rabbit anti-STAT3 (Catalog# 12640), rabbit anti-pY705 STAT3 (Catalog# 9145S), (Cell Signaling Technology, Danvers, MA, USA), anti-NFκB p65 antibody (Catalog# ab32536, Abcam, UK), anti-Glutathione Peroxidase 3/GPX-3 antibody (Catalog# ab256470, Abcam, UK) and anti-GAPDH antibody (Catalog# ab8245, Abcam, UK). Goat anti-rabbit polyclonal immunoglobulin conjugated with horseradish peroxidase (Cell Signaling Technology Inc., MA, USA) was used as a secondary antibody (1:5000) in blocking buffer. Bands were visualized by chemiluminescence, using an enhanced chemiluminescence kit (ECL, GE Healthcare, Chicago, IL, USA. Protein bands were evaluated densitometrically relative to GAPDH using Image J Software [28, 29].

**Histopathological Examination**

After sacrifice; part of each ventricle was prepared for histopathological examination, dissected, fixed in formaldehyde for 24 h and embedded in paraffin wax. Five µm sections were cut for hematoxylin and eosin stain and immunohistochemistry. The pathologist examined these slides in a blinded fashion using light microscopy (Olympus microscope, Japan). Scoring of the histopathological changes in different groups was assessed according to the degree of vascular congestion; hemorrhage; hemosiderosis; muscle striation; apoptosis and necrosis. The grades were as follows: score − normal, score + mild, score ++ moderate and score +++ severe [3].

**Immunohistochemistry**

Slides of the cardiac tissue specimens were de-paraffinized with xylene, hydrated in a descending graded ethyl alcohol then treated by 3% hydrogen peroxide for 30 min to block endogenous peroxides. Slides were washed in PBS solution and boiled for 20 min in citrate buffer (pH 6.0) by microwave for antigen retrieval [1, 3].

After rinse in PBS, caspase3 antibody was applied and incubated overnight then rinsed in PBS before applying the biotinylated secondary antibody for 30 min. The streptavidin–biotin complex reagent was added for 30 min after washing in PBS. For detection of brown color; 3, 3-diaminobenzidine hydrochloride (DAB) was used. Slides were
washed in distilled water, counterstained with hematoxylin, dehydrated in ethanol, cleared in xylene and covered slipped. The mean area percentage of active caspase 3 reaction was measured [30].

Statistical Analysis

Data of current model were analyzed using one-way ANOVA then Tukey’s multiple comparison test. The values were expressed as Mean ± SEM using GraphPad Prism software (version 5) for statistical analysis. The differences were considered as significant results when the $P$ value < 0.05.

Results

Effect of DAP on Serum Cardiac Enzymes (Troponin I, CK-MB, LDH)

Cd (5 mg/kg/day) oral administration for 7 days led to significant increase in serum cardiac enzymes level (troponin I, CK-MB, LDH) compared to control group. Co-administration of DAP significantly ameliorated this effect compared to Cd given group alone in dose dependent manner (Table 1).

Effect of DAP on the Evaluated Oxidative Stress Parameters (MDA, GSH, HO1, Nrf2 and TAC) in Cd Induced Cardiotoxicity

Cd group significantly increased MDA but decreased GSH, HO1, Nrf2 and TAC compared to control group. DAP plus Cd given groups significantly decreased MDA but increased GSH, HO1, Nrf2 and TAC compared to Cd administered group alone. High dose of DAP was more protective than lower doses (Table 2).

Effect of DAP on Heart Weights, TLR2, IL6 in Cd Induced Cardiotoxicity

ELISA measurement of TLR2, IL6 showed a significant increase in their tissue levels in Cd given group compared to control group. However, co-administration of DAP could...
Fig. 3 Histopathological evaluation results. 

(a) Photomicrographs of myocardial sections of control group shows striated branching cardiac muscle fibres with acidophilic cytoplasm and central oval vesicular nucleus (arrows) separated by blood capillaries (C).

(b) and (c) longitudinal and cross myocardial sections of cadmium cardiotoxic group show fragmented necrotic cardiac muscle fibers (*). Some fibers have pyknotic nuclei and hyperacidophilic cytoplasm (black arrows), marked congested dilated blood capillary (C), inflammatory cells infiltration (circles) and hemorrhage (H).

(d) represents low dose dapagliflozin treated group that shows fragmented necrotic pale stained cardiac muscle fibers (*), some fibers have pyknotic nuclei and hyperacidophilic cytoplasm (black arrows), marked congested dilated blood capillary (C) and inflammatory cells infiltration (circles).

(e) represents moderate dose dapagliflozin treated group showing cardiac muscle fibres appear more or less normal, still few fibres have pyknotic nuclei (arrows). Moreover, mild congested blood capillaries (C) and few scattered inflammatory cells (circle) are noticed.

(f) of high dose dapagliflozin treated group showing cardiac muscle fibres appear more or less normal, still few scattered inflammatory cells (circle) are noticed [H&E, ×400]
significantly ameliorate these changes in comparison to Cd cardiotoxic group in dose dependent manner and high dose was more protective than lower doses (Table 3).

Heart weights significantly increased in Cd cardiotoxic group compared to control group. On the contrary, Cd plus DAP showed significant decrease in heart weight compared to Cd given group.

**Western Blotting Results for Measuring TNFα, NFκB, GPx and P-STAT3**

Results revealed a significant increase of TNFα and NFκB but a decrease of GPx and P-STAT3 level in Cd cardiotoxic group compared to control one but co-administration of DAP decreased the tissue level of TNFα and NFκB but increased GPx and P-STAT3 compared to Cd given group alone (Figs. 1a, b, 2a, b).

**Histopathological Evaluation Results (Fig. 3)**

Myocardial sections of control group showed normal structure with striated branching cardiac muscle fibres, acidophilic cytoplasm, central oval vesicular nuclei and the cardiomyocytes were separated by blood capillaries (Fig. 3a). The longitudinal and cross myocardial sections of Cd cardiotoxic group showed fragmented necrotic cardiac muscle fibres, some fibres had pyknotic nuclei and hyperacidophilic cytoplasm (apoptotic morphology). Moreover, there were markedly congested and dilated blood capillaries. Inflammatory cell infiltration and areas of hemorrhage were also noticed (Fig. 3b, c). In the low dose DAP treated group, necrotic pale stained cardiac muscle fibers, scattered fibers with apoptotic morphology (pyknotic nuclei and hyperacidophilic cytoplasm), dilated blood capillary and inflammatory cell infiltration were also evident (Fig. 3d). Moderate dose DAP treated group showed the cardiac muscle fibres with more or less normal features but still few fibres had pyknotic nuclei. Also, mild congested blood capillaries and few scattered inflammatory cells were observed (Fig. 3e). Interestingly, there was marked improvement in high dose DAP treated group as the cardiac muscle fibres appeared more or less normal with few scattered inflammatory cells in-between (Fig. 3f). These data were supported by scoring of the histopathological findings (Table 4).

**Evaluation of Cleaved Caspase3 Immunoexpression (Fig. 4)**

Sections of control heart tissue, immunostained with activated caspase3 showed negative expression either in the cardiomyocytes or endothelial lining of the blood capillaries. Heart tissues of Cd cardiotoxic group had high expression in the cytoplasm of the cardiomyocyte and the endothelial lining of the blood capillaries. Moderate expression was also noticed in the cytoplasm of the cardiomyocyte and the endothelium of the low dose DAP treated group. In the moderate dose DAP treated group, the expression was faint and localized to few cardiomyocytes, although most of the capillary endothelium showed positive expression. The cardiomyocytes of the high dose DAP treated group showed negative expression, while few endothelium cells showed positive expression.

**Semiquantitative Analysis of Cleaved Caspase3 Immunoexpression (Fig. 4f)**

Results showed a significant increase of cleaved caspase3 immunooexpression in Cd cardiotoxic group compared to control group. However, there was a significant decrease in its immunooexpression in DAP co-administered groups in dose dependent manner.

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**Table 4 Scoring of the histopathological changes**

| Histological changes                             | CON | Cd     | Cd+DAP-LD | Cd+DAP-MD | Cd+DAP-HD |
|--------------------------------------------------|-----|--------|-----------|-----------|-----------|
| Disruption of cardiac muscles architecture       | −   | ++++   | ++        | +         | +         |
| Loss of muscular striations                      | −   | ++++   | +++       | ++        | +         |
| Vascular congestion                              | −   | ++++   | +         | −         | −         |
| Interstitial hemorrhage and hemosiderin deposition| −   | ++++   | +         | −         | −         |
| Inflammatory cellular                            | −   | +      | +         | +         | +         |
| Apoptosis                                        | −   | +      | +         | +         | +         |
| Necrosis                                         | −   | +++    | ++        | +         | +         |

Scoring of the histopathological changes in different groups. The structural changes of tissue were assessed according to the degree of vascular congestion; hemorrhage; hemosiderosis; muscle striation; apoptosis and necrosis. The severity of the lesions was graded as follows: score − was considered normal, score + mild, score ++ moderate, and score +++ severe.
Discussion

Cd is a well-known environmental contaminant, cardiototoxic, and carcinogenic heavy metal [2, 31]. Our model is the first step in exploring the cardioprotective properties of DAP in Cd induced cardiac injury with studying of the different mechanisms that mediate such effect. Cd induced cardiac injury was detected in our results in form of significant increase of cardiac enzymes, heart weights, MDA, TLR2, IL6, caspase3, TNFα, NFXB but significant
Cd cardiotoxicity. Nrf2 is kept inactive in the cytoplasm and to keep the cellular integrity and prevent the excessive released free radicals [5, 19, 29, 37–40]. The antioxidative, GSH, decreases due to interaction with the excessively released free radicals (arrow), (active caspase3 immunohistochemistry ×400). f Semiquantitative analysis of cleaved caspase3 immunoeexpression. Results showed significant increase in its immunoeexpression in cadmium given group in comparison to normal control group. However, dapagliflozin plus cadmium showed significant decrease in cleaved caspase3 immunoeexpression compared to cadmium administered group alone. Values are representation of 8 observations as Mean ± SEM. Results are considered significantly different when p < 0.05. Significant difference compared to control. bSignificant difference compared to cadmium given group. cSignificant difference compared to dapagliflozin high dose (10 mg/kg/day) plus cadmium given group.

Indiscriminate exposure to Cd causes generation of free radicals and oxidative dysfunction that leads to lipid peroxidation. This associates with alteration in the antioxidant defense mechanism followed by oxidative injury of proteins, DNA, and lipids that is found in our model as significant increase in MDA levels; the most reliable marker for evaluating oxidative stress and lipid peroxidation [32–36]. In addition, other oxidative stress markers including lipid hydroperoxide and protein carbonyls increased in Cd induced toxicity and they have a critical role in mediating such injury.

Antioxidant enzymes are considered as the first line of defense against the oxidative challenges to protect cell membrane and to keep the cellular integrity and prevent the pathogenesis of different degenerative diseases. Superoxide is converted by superoxide dismutase to hydroxyl radicals and hydrogen peroxide then GPx utilizes GSH to reduce hydrogen peroxide with formation of oxidized glutathione and H₂O₂. During oxidative stress, the level of enzymatic antioxidant enzymes notably GPx as well as the non-enzymatic antioxidant, GSH, decreases due to interaction with the excessively released free radicals [5, 19, 29, 37–40]. The same was observed in our results as there is general reduction in the measured GSH, GPx and TAC in Cd treated rats and this is in accordance with previous studies [31, 39–42].

Nrf2/HO1 pathway is also highly essential in evaluating Cd cardiotoxicity. Nrf2 is kept inactive in the cytoplasm binding with keap under healthy conditions. However, upon stimulation of oxidative stress, it travels into the nucleus and attaches to its DNA sequence followed by stimulation of gene transcription of several antioxidants including HO1 that catalyzes the rate-limiting step in the process of heme catabolism leading to anti-inflammatory, anti-oxidant, anti-apoptotic effects; it also controls cell proliferation and differentiation [43]. Our model reveals significant downregulation of Nrf2/HO1 pathway and the same was detected with others [43, 44].

Oxidative stress induces cell membrane lipid peroxidation and damage with release of the intracellular cardiac enzymes and increasing their serum level including troponin I, CK-MB and LDH. Troponin I is one of the contractile cardiac apparatus that regulates sliding of actin over myosin. It is a specific diagnostic marker for detecting cardiac injury. Moreover, CK-MB is widely distributed in skeletal muscles all over the body and highly sensitive to the occurrence of cardiac injury [18, 34–36]. This is in accordance with our data that showed marked increase in troponin I, CK-MB and LDH in Cd cardiotoxic group.

Initiation of inflammation is another essential signaling cascade in mediating Cd cardiotoxicity. We evaluated two essential pathways; IL6/STAT3 and TLR2/TNFα which have great role in mediating different tissue injuries. We found significant disturbance of both pathways in Cd given group reflecting occurrence of inflammation. In addition, JAK/STAT signaling cascade is considered as a critical inflammatory pathway in cardiac injury that stimulates release of other inflammatory and apoptotic mediators including TNFα and NFκB [45, 46]. One of STATs family is STAT3 which is controlled by IL6 and highly expressed in many heart cells including cardiomyocytes, fibroblasts, smooth muscle cells, endothelial cells, and inflammatory cells. Disturbances in IL6/STAT3 pathway could enhance Cd induced cardiac damage. Moreover, STAT3 is a crucial regulator of various intracellular events and it can regulate autophagy by promoting its mitochondrial localization. However, downregulation of STAT3 leads to cell apoptosis and damage. Notably, recent studies have been carried out based on the cardioprotective effect of STAT3 [46–48]. Besides that, there is another inflammatory signaling pathway; TLR2/TNFα that has an essential role in Cd induced toxicities. Toll-like receptors enhance further release of TNFα and other interleukins followed by activation of an important inflammatory and apoptotic molecule; NFκB which is highly involved in Cd induced cardiotoxicity [2, 10–13, 49]. This is in accordance with our current findings that showed significant increases of TNFα, NFκB and IL6 in Cd given group compared to normal control group.

Occurrence of oxidative stress and inflammation is followed by stimulation of the apoptotic cascade that is
Several studies found that this drug group could ameliorate cardiac morphologic changes including cardiac hypertrophy, fibrosis, heart failure and decrease the infarcted size. Moreover; DAP could improve systolic and diastolic LV function in cases of diabetic cardiomyopathy and control cardiac arrhythmia in I/R injury. Furthermore, they decreased cardiac preload and afterload with diminishing intracellular Na+ and Ca2+ loading that is mostly reflected in heart failure [20].

This in agreement with our findings which reveal that co-administration of DAP could markedly ameliorate Cd induced biochemical and histopathological changes. Our results found significant decrease and normalization of cardiac enzymes level including serum CK-MB, LDH and Troponin I. Moreover, there is marked regulation of oxidant/antioxidant imbalances including oxidative stress parameter, MDA level but increases tissue anti-oxidants as GSH and GPs, with elevation of the whole the TAC causing protection of the cell membrane and preventing release of cardiac enzymes. In addition, DAP succeeded in modulating IL6/STAT3 and TLR2/TNFα pathway reflecting its ability to suppress both inflammatory and apoptotic processes as found in previous models [20, 56, 57]. Caspase3; an essential apoptotic marker, decreased significantly on co-administration of DAP with Cd in comparison to Cd given group alone confirming its anti-apoptotic effect with marked improvement of the histopathological features.

This cardioprotective properties of DAP was already discussed in other models of cardiac injury including diabetic cardiomyopathy, ischemia reperfusion models, doxorubicin cardiotoxicity, myocardial infarction and heart failure [20, 21, 57–59]. These effects were based upon anti-oxidant, anti-inflammatory and anti-apoptotic properties of DAP and its ability to regulate renin angiotensin aldosterone system and keep the myocardium. Our model may give a promise to consider DAP as cardiac protector against Cd cardiotoxicity.

**Conclusion**

Dapagliflozin could significantly reduce cadmium cardiotoxicity by various mechanisms including anti-oxidant, anti-inflammatory and anti-apoptotic properties via modulating IL6/STAT3 and TLR2/TNFα pathways. Further studies are highly encouraged to evaluate the cardioprotective role of dapagliflozin in cadmium cardiotoxic patients.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s12012-022-09768-0.

**Author Contributions** Dr. MMR and Dr. SS selected the point, performed the experimental part, wrote the manuscript and sent it for publication. Dr. RAR performed and wrote the histopathology and immunohistochemistry. Dr. MAF performed and wrote the part of
western blotting analysis. All authors revised the final version of the manuscript and agreed for publishing it.

**Funding** Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB). This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Data Availability** All data are available in prism files as supplementary material.

**Code Availability** Not applicable.

**Declarations**

**Competing interests** The authors declare no competing interests.

**Ethical Approval** Animal handling, medications, and animal sacrifice were carried out according to the guidelines of the experimental animals care and approved by the Institutional Ethical Committee, Faculty of Medicine, Minia University, Egypt in agreement with the NIH Guide for taking care and use of laboratory animals. Approval No. 20:3/2021.

**Informed Consent** Not applicable.

**Research Involving Human Participants and/or Animals** Animals.

**Consent to Participate** Not applicable.

**Consent for Publication** Not applicable.

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