Original Article

# A Comparative Histological and Immunohistochemical Study on the Possible Therapeutic Effects of Empagliflozin and platelet-rich plasma Against Cisplatin Induced Cardiotoxicity in Rats

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## ABSTRACT

**Introduction and Objectives:** Cisplatin is a highly effective chemotherapeutic drug used to treat various cancers. However, cardiotoxicity is one of the most important adverse effects of it. This study aimed to investigate and compare possible therapeutic effects of empagliflozin (EMPA) and platelet-rich plasma (PRP) on cisplatin induced cardiotoxicity in adult male albino rats.

**Material and Methods:** 48 adult male albino rats were classified into: Group I (control group) and Group II (Experimental group). All rats in group II were injected once with cisplatin (10 mg/kg) intraperitoneally (ip) then after 7 days they were divided into; cisplatin subgroup(II-a); rats were sacrificed on the 7$^{th}$ day, spontaneous recovery subgroup (II-b) was left untreated for 3 weeks, EMPA-treated subgroup (II-c) received EMPA orally daily (20 mg/kg/day) for 3 weeks and PRP-treated subgroup (II-d) received 0.5 ml/kg PRP subcutaneously (SC) twice weekly for 3 weeks. At the end of the experiment, Blood samples were collected to measure creatine kinase –MB (CK –MB), cardiac troponin I (cTnI), Superoxide Dismutase (SOD) and malondialdehyde (MDA). Cardiac muscle specimens were acquired for histological, immunohistochemical, morphometric and statistical analysis.

**Results:** Mean serum levels of CK-MB, cTnI & MDA was significantly increased associated with significant decrease of SOD level in cisplatin & spontaneous recovery subgroups (Subgroups II-a & II-b respectively). In addition, area percentage of collagen fibers, caspase-3 & fibronectin showed a significant increase and area percentage of Connexin 43 (Cx43) showed a significant decrease in subgroups II-a & II-b. While, EMPA-treated & PRP-treated subgroups (Subgroups II-c & II-d respectively) encountered significant amelioration in biochemical markers, histological & immunohistochemical changes induced by cisplatin with a more obvious improvement in Subgroup II-d.

**Conclusion:** EMPA and PRP significantly improved cisplatin-induced cardiac damage by alleviating oxidative stress, suppressing inflammation and inhibiting apoptosis; with a more pronounced improvement in response to PRP than EMPA.

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**Key Words:** Cardiotoxicity, cisplatin, empagliflozin, PRP.

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## INTRODUCTION

Cisplatin is a highly effective chemotherapeutic agent used to treat different types of cancer, such as testicular, ovarian, lung, urinary bladder, head and neck cancer[1]. Cisplatin-induced cardiotoxicity is one of its most significant limiting adverse effects characterized by echocardiography changes, arrhythmias, angina and acute myocardial infarction, myocarditis, cardiomyopathy and congestive heart failure[2]. The mechanism of cisplatin induced cardiotoxicity could be due to the direct toxic effects of cisplatin on cardiac muscle cells, or the oxidative stress caused by the production of reactive oxygen species (ROS) and transition to a prothrombotic state[3].

Empagliflozin, an inhibitor of sodium glucose cotransporter 2 (SGLT2i), is a newly developed oral antidiabetic drug that improves kidney excretion of glucose and reduces hyperglycemia in non-insulin-independent mechanism by inhibiting SGLT2 in a highly selective manner[4]. In addition to its antihyperglycemic action, SGLT2i has a protective effect on the cardiovascular system in terms of reducing hospitalizations for heart failure and reducing cardiovascular mortality in patients suffering from type II diabetes mellitus (DM)[5]. Ongoing clinical studies are being conducted to assess the SGLT2i's cardiovascular effects in patients with non-diabetic heart failure. A recent view has hypothesized that the advantage of SGLTis in heart failure (HF) could be mediated by the sodium hydrogen exchanger (NHE) rather than the effect on glucose reabsorption; however, a complete mechanical explanation of the SGLTi extrarenal cardioprotective effects remain unclear[6].

Platelet-rich plasma (PRP) is defined as autologous platelet concentration which is 3-5 times higher than the physiological concentration of platelets in the blood[7]. PRP therapy is a relatively new method of regenerative medicine and has attracted a lot of attention over the past two decades[8]. The PRP possible therapeutic effect is due to several cytokines as well as growth factors such as platelet

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factor 4, platelet derived growth factors, transforming growth factors β1, insulin-like growth factors, fibroblast growth factor 2, and vascular endothelial growth factor (VEGF)[9]. These factors are proved to enhance epithelial and endothelial regeneration, stimulate angiogenesis and cell differentiation, improve hemostatic response, increase collagen synthesis, promote cell migration and soft tissue healing[10].

This study aimed to investigate and compare the possible therapeutic effects of empagliflozin and PRP on cisplatin induced cardiotoxicity in adult male albino rats. The effectiveness of empagliflozin and PRP therapies was monitored by serological, histological, immunohistochemical and morphometric studies.

## MATERIALS AND METHODS

### *Drugs*

**Cisplatin:** was purchased from Hikma specialized pharmaceuticals, Badr city, Cairo, A.R.E. The drug is in the form of vials (Unistin); each contains 10 mg/10ml.

**Empagliflozin (EMPA):** was provided as 10 mg tablets by Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany.

**Platelet-rich Plasma (PRP):** was freshly prepared in the laboratory of Biochemistry Department, Faculty of Medicine, Cairo University. Using completely sterile techniques, the rats were anesthetized with Ketamine, then venous blood was drawn from rat's tail veins and conveyed into test tubes containing 3.2% sodium citrate (Merck, Darmstadt, Germany) at a blood/citrate ratio of 9/1. Centrifugation of blood was done using a 'soft' spin for 6 minutes at 1480 rpm to isolate erythrocytes. Platelets with supernatant plasma were transferred to another sterile tube without anticoagulants. The tube was centrifuged again using a 'hard' spin for 15 min at a rapid speed of 3400 rpm to obtain a platelet concentrate. The platelet-poor plasma (PPP) in the upper 2/3 of the tube was removed, while PRP was considered in the remaining lower (1/3). The PRP was isolated, dissolved in phosphate buffer saline (PBS) (Sigma Chemical CO. P. 3813 USA) and frozen at -20°C for subsequent use[11].

### *Animals*

This study was conducted on 48 adult male albino rats, aged about 12 weeks and had an average body weight of 200 grams. The animals were purchased and bred in the Animal House of Kasr Al Ainy Hospital (Faculty of Medicine, Cairo University). Animals were housed in hygienic cages, kept in clean well-ventilated rooms and allowed free access of standard diet and water. The study was performed according to the ethical policies and standards of the Animal Ethics Committee, Cairo University.

### *Experimental design*

The rats were divided randomly into 2 main groups:

**Group I** (Control group), 16 rats: rats were injected intraperitoneally (ip) on the first day of the experiment with 2 ml of 0.9% NaCl solution (the vehicle of cisplatin) and then on the 7th day, rats were randomly subdivided into 4 subgroups:

- Subgroup I-a, 4 rats: rats were sacrificed on the 7th day of the experiment.
- Subgroup I-b, 4 rats: rats were sacrificed at the end of the experiment (after 4 weeks).
- Subgroup I-c, 4 rats: on the 7th day of the experiment, each rat received 4 ml/day of drinking water (the solvent of EMPA) once daily for 3 weeks by the oral route via a syringe without its needle.
- Subgroup I-d, 4 rats: on the 7th day, each rat was injected with 0.1 ml of PBS (the vehicle of PRP) subcutaneously twice weekly for 3 weeks.

Rats of subgroups I-b, I-c & I-d were scarified at the end of the experiment (after 4 weeks).

**Group II** (Experimental group), 32 rats: each rat was injected with a single ip dose of cisplatin (10 mg/kg) on the first day of the experiment. On the 7th day, rats were randomly subdivided into 4 subgroups:

Cisplatin subgroup (Subgroup II-a), 8 rats: rats were sacrificed on the 7th day of the experiment to ensure cardiac damage[12].

Spontaneous recovery subgroup (Subgroup II-b), 8 rats: rats were left untreated for 3 weeks to assess spontaneous recovery.

EMPA treated subgroup (Subgroup II-c), 8 rats: each rat received EMPA as a single daily dose of 4 ml of water containing 4 mg EMPA (20 mg/kg/day)[5] for 3 weeks from the 7th day till the end of the experiment. The dose was administered via a syringe without its needle.

PRP treated subgroup (Subgroup II-d), 8 rats: each rat received 0.1 ml of PBS containing 0.1 ml of PRP subcutaneously twice weekly for 3 weeks from the 7th day till the end of experiment (0.5 mL/kg 1:1 in PBS)[13].

At the end of the experiment (after 4 weeks from the start of the experiment), rats of subgroups II-b, II-c & II-d were sacrificed.

NB: In the control and experimental groups, the doses were calculated for a 200 mg rat.

### *Biochemical study*

Before sacrifice, blood samples were drawn from tail veins in heparinized capillary tubes. Serum creatine kinase –MB (CK –MB) and cardiac troponin I (cTnI) levels; markers of cardiac muscle damage[14] were measured. In addition, the antioxidant biomarker [Superoxide Dismutase (SOD)] and the oxidative stress biomarker [malondialdehyde (MDA)][15] were estimated in the Biochemistry Department, Faculty of Medicine, Cairo University.

### Histological study

Under lethal dose of general anesthesia, rats were sacrificed by ip injection of 100 mg/kg ketamine-xylazine[16]. A ventral midline incision was performed, and hearts were excised, washed in saline and fixed for 48 hours in 10% formol saline. Cardiac muscle specimens were dissected out from the apex of the left ventricle and processed in 5-7 µm paraffin sections. Sections were submitted to:

a. H & E stain[17].

b. Mallory's trichrome stain for collagen fibers[18].

c. Immunohistochemical staining using the avidin-biotin peroxidase complex technique[19] for:

   1. Anti-Connexin 43 (Cx43) antibody as a marker for gap junctions of intercalated discs. It is a ready-to-use rabbit monoclonal antibody (Santa Cruz Biotechnology, Inc, Europe (C-20) sc 6560 Ab).

   2. Anti-Caspase 3 antibody as a marker of apoptosis. It is ready-to-use rabbit polyclonal Caspase 3 (CPP32) Ab-4 (NEOmarkers (Thermo scientific) Laboratories (USA), catalogue number RB-1197-R7).

   3. Anti-Fibronectin antibody as a marker for fibronectin deposition in myocardial cells & extracellular matrix (ECM). It is a ready-to-use mouse monoclonal antibody (Santa Cruz Biotechnology, Inc, Europe (EP5) sc-8422 Ab).

Briefly[19], sections were de-paraffinized in xylene, rehydrated with descending grades of alcohol. Blocking endogenous peroxidase activity was done by submerging the sections in $H_2O_2$ for 15 min. The sections were incubated for 5 min with 2 drops of Ultra V Block to block non-specific background followed by incubation with 2 drops of the primary antibody for 60 min. Then incubate the slides for 10 min in 2 drops of biotinylated goat anti-polyvalent secondary antibody then for 10 min with 2 drops of streptavidin-peroxidase. The reaction was visualized with; Ultravsion One detection system, HRP polymer and diaminiobenzide (DAB) Plus Chromogen. Counterstaining of the slides were done with Mayer's hematoxylin, dehydrated by ascending alcohol concentrations, purified with xylene and mounted.

The positive (+ve) control for Cx43 was the normal cardiac tissue with cytoplasmic reaction. The positive (+ve) control for caspase 3 was the human tonsil sections and the reaction was cytoplasmic, while that of fibronectin was human aorta tissue with ECM and cytoplasmic reaction. On the other hand, one of the cardiac muscle sections was used as –ve control by passing the steps without applying the 1ry antibody.

### Morphometric Study

Results were acquired using "Leica Qwin 500C" image analyzer computer system (Leica Imaging System Ltd, Cambridge, UK) in the Medical Histology and Cell Biology department, Faculty of medicine, Cairo university. Light microscope was used to examine the slides and the parameters were measured in 10 non-overlapping randomly selected high-power fields (x400) for each section using the binary mode:

a. The mean area % of collagen fibers in Mallory's Trichrome Stained Sections.

b. The mean area % of anti-Cx43, anti-caspase 3 & anti-fibronectin positive immunoreactivity in immunostained sections.

### Statistical Analysis

Quantitative data were summarized as means ± standard deviation (SD) and compared using one-way analysis-of-variance (ANOVA) followed by post hoc Tukey test on Statistical Package for Social Science (SPSS) software, version 16. Differences were considered statistically significant at a *"p" value* < 0.05[20].

## RESULTS

During the experiment, no deaths were observed in rats of all groups. The biochemical, histological and immunohistochemical results of all control subgroups were similar. So, they were referred to as control group (Group I).

### Biochemical results

The mean values of serum levels of CK-MB, cTnI & MDA showed a statistically significant increase in the cisplatin & spontaneous recovery subgroups (II-a & II-b) respectively in comparison to the other subgroups. In addition, EMPA-treated subgroup (II-c) showed a statistically significant increase compared to the control & PRP-treated subgroup (II-d). On the other hand, the mean values of serum SOD level showed a statistically significant decrease in (cisplatin & spontaneous recovery) subgroups in comparison with the other subgroups. Moreover, EMPA-treated subgroup (II-c) showed statistically significant decrease compared to control groups & PRP-treated subgroup (II-d). No significant difference was observed between the control & PRP-treated subgroup (II-d) (Table 1).

### Histological Results

**(a) Hematoxylin and Eosin-Stained Sections**

Longitudinal sections & transverse sections of the apex of the heart of all groups were examined in (Figures 1,2) respectively.

Sections in rat cardiac muscle of the control group exhibited normal muscle fibers showing acidophilic sarcoplasm with transverse striations, centrally located vesicular oval nuclei, intercalated discs and clear

sarcolemma. Small blood vessels were seen in between the muscle fibers (Figures 1a,2a).

Cisplatin subgroup (Subgroup II-a) exhibited degeneration, discontinuation and wide separation of cardiac muscle fibers with focal areas of deeply acidophilic sarcoplasm, loss of striations and dissolved sarcolemma. Darkly stained pyknotic nuclei and blood extravasation were also observed (Figures 1b,2b).

Spontaneous recovery subgroup (Subgroup II-b) revealed cardiac myocytes degeneration with darkly stained pyknotic nuclei, dissolved sarcolemma and coarse endomysium in between. Focal areas of sarcoplasm of various muscle fibers appeared either deeply acidophilic or vacuolated. Blood extravasation and moderate cellular infiltration were also seen (Figures 1c,2c).

EMPA-treated subgroup (Subgroup II-c) exhibited many well-organized muscle fibers with transverse striations, intact sarcolemma and intercalated discs. Some fibers have central pale vesicular nuclei and others have dark pyknotic nuclei. Congested blood vessels and small areas of mild cellular infiltration were also observed (Figures 1d,2d).

PRP-treated group (Subgroup II-d) showing nearly normal cardiac muscle fibers separated by fine endomysium. Most of fibers have vesicular central nuclei, acidophilic sarcoplasm with transverse striations, intercalated discs and clear sarcolemma. Few fibers have dark pyknotic nuclei. Small blood vessels were observed between the muscle fibers (Figures 1e,2e).

**(b) Mallory's Trichrome Stained Sections**

Sections of the control group showed fine collagen fibers between the cardiac muscle fibers (Figure 3a). However, cisplatin subgroup (II-a) revealed increased amount of collagen fibers between the muscle fibers compared to the control (Figure 3b). A marked deposition of irregular collagen fibers between muscle fibers was found in spontaneous recovery subgroup (II-b) (Figure 3c). EMPA-treated subgroup (II-c) showed reduced amount of collagen fibers between the muscle fibers (Figure 3d) compared to subgroups II-a and II-b, while PRP-treated subgroup (II-d) exhibited minimal amount of collagen fibers between the muscle fibers (Figure 3e).

**(c) Immunohistochemical Results**

**1- Anti-Connexin 43 immunostained sections**

Sections of group I showed multiple positive Cx43 immunoreactivity in intercalated discs between cardiomyocytes (Figure 4a). Cisplatin subgroup (II-a) showed few positive Cx43 immunoreactivity in the disrupted muscle fibers with disrupted discs in many fibers (Figure 4b). While in spontaneous recovery subgroup (II-b), few disrupted immunoreactive intercalated discs were observed among the disrupted fibers (Figure 4c). Sections from EMPA-treated subgroup (II-c) showed some positive Cx43 immunoreactivity (Figure 4d), while PRP-treated subgroup (II-d) showed multiple positive Cx43 immunoreactivity in intercalated discs (Figure 4e).

**2- Anti-Caspase 3 immuno-stained sections**

Transverse sections in the cardiac muscle of group I showed polyhedral fibers with localized sarcoplasmic immunostaining by anti-caspase-3 antibody (Figure 5a). A strong sacroplasmic immunostaining was detected in many cardiac muscle fibers in cisplatin subgroup (II-a) & in most of fibers in spontaneous recovery subgroup (Subgroup II-b) (Figures 5b,c respectively). However, EMPA-treated subgroup (II-c) exhibited mild sarcoplasmic immunostaining in some muscle fibers (Figure 5d) and PRP-treated subgroup (II-d) showed minimal sarcoplasmic immunostaining in few fibers (Figure 5e).

**3- Anti-Fibronectin immunostained sections**

Sections from control rats exhibited negative immunoreaction for fibronectin antibody (Figure 6a). In cisplatin subgroup (subgroup II-a), there was positive immunoreactivity in the sarcoplasm of many myocardial cells and focal areas of ECM (Figure 6b). However, spontaneous recovery subgroup (II-b) showed a strong positive and wide distributed immunoreactivity in both myocardial cells and ECM (Figure 6c). EMPA-treated Subgroup (II-c) displayed a mild to moderate positive immunoreactivity in few myocardial cells and limited areas of ECM (Figure 6d), while PRP-treated subgroup (II-d) revealed very weak immunoreactivity in both myocardial cells and ECM (Figure 6e).

## *Morphometric results*

In both cisplatin & spontaneous recovery subgroups (II-a &II-b) respectively, the mean area % of collagen fibers, anti-caspase 3 & anti-fibronectin immunoreactivity showed a statistically significant increase in comparison with all other subgroups. In addition, a statistically significant increase was detected in EMP- treated subroup (II-c) when compared to the control & PRP- treated subgroup (II-d). Moreover, the mean area % of anti-Cx43 immunoreactivity revealed a statistically significant decrease in cisplatin & spontaneous recovery subgroups (II-a &II-b respectively) compared to all other subgroups and also in EMPA-treated subgroup (II-c) compared to the control & PRP-treated subgroup (II-d). In addition, there was a satistically significant difference between cisplatin & spontaneous recovery subgroups (II-a &II-b respectively), as mean area % of collagen fibers, anti-caspase 3 & anti-fibronectin immunoreactivity was significantly increased associated with significant decrease in mean area % of anti-Cx43 immunoreactivity in spontaneous recovery subgroup (II-b). However, there was no statistically significant difference between the control & PRP-treated subgroup (II-d) except in the the area % of fibronectin (Table 2).

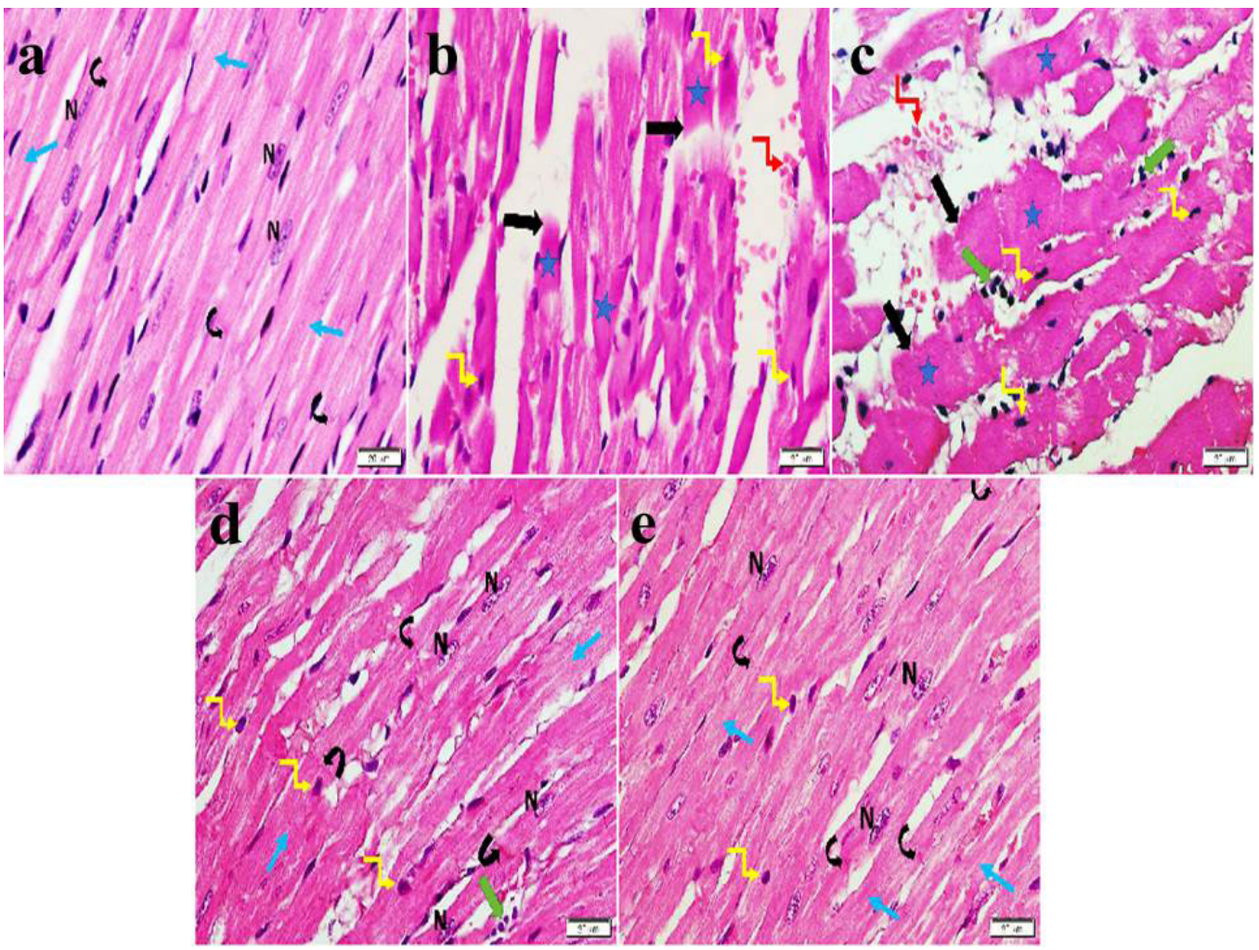


**Fig. 1:** Photomicrographs of longitudinal sections in the apex of the left ventricle (H&E, X400).

**a:** The control groups (I) show normal muscle fibers exhibiting acidophilic sarcoplasm with transverse striations (blue arrows), central vesicular oval nuclei (Letter N) and intercalated discs (curved arrows).

**b:** cisplatin subgroup (II-a) shows disrupted, degenerated and widely separated muscle fibers (arrows) with focal areas of deeply acidophilic sarcoplasm and lost striations (blue stars). Some fibers show darkly stained pyknotic nuclei (yellow kinked arrows). Extravasated RBCs (red kinked arrow) are also seen.

**c:** Spontaneous recovery subgroup (II-b) reveals disrupted, degenerated and widely separated muscle fibers (black arrows) with darkly stained pyknotic nuclei (yellow kinked arrows). Focal areas of the sarcoplasm appear deeply acidophilic with lost sriations (blue stars) and others appear vacuolated (Letter V). Extravasated RBCs (red kinked arrow) and moderate cellular infiltration (green arrows) are also seen.

**d:** EMPA-treated subgroup (II-c) shows many well-organized muscle fibers with transverse striations (blue arrows) and intercalated discs (curved arrows). Some fibers have central pale vesicular nuclei (Letter N) and others have dark pyknotic nuclei (yellow kinked arrows). Notice the presence of mild cellular infiltration (green arrow).

**e:** PRP-treated subgroup (II-d) shows nearly normal cardiac muscle fibers with vesicular central oval nuclei (Letter N), acidophilic sarcoplasm with transverse striations (blue arrows) and intercalated discs (curved arrows). Few fibers have dark pyknotic nuclei (yellow kinked arrows).

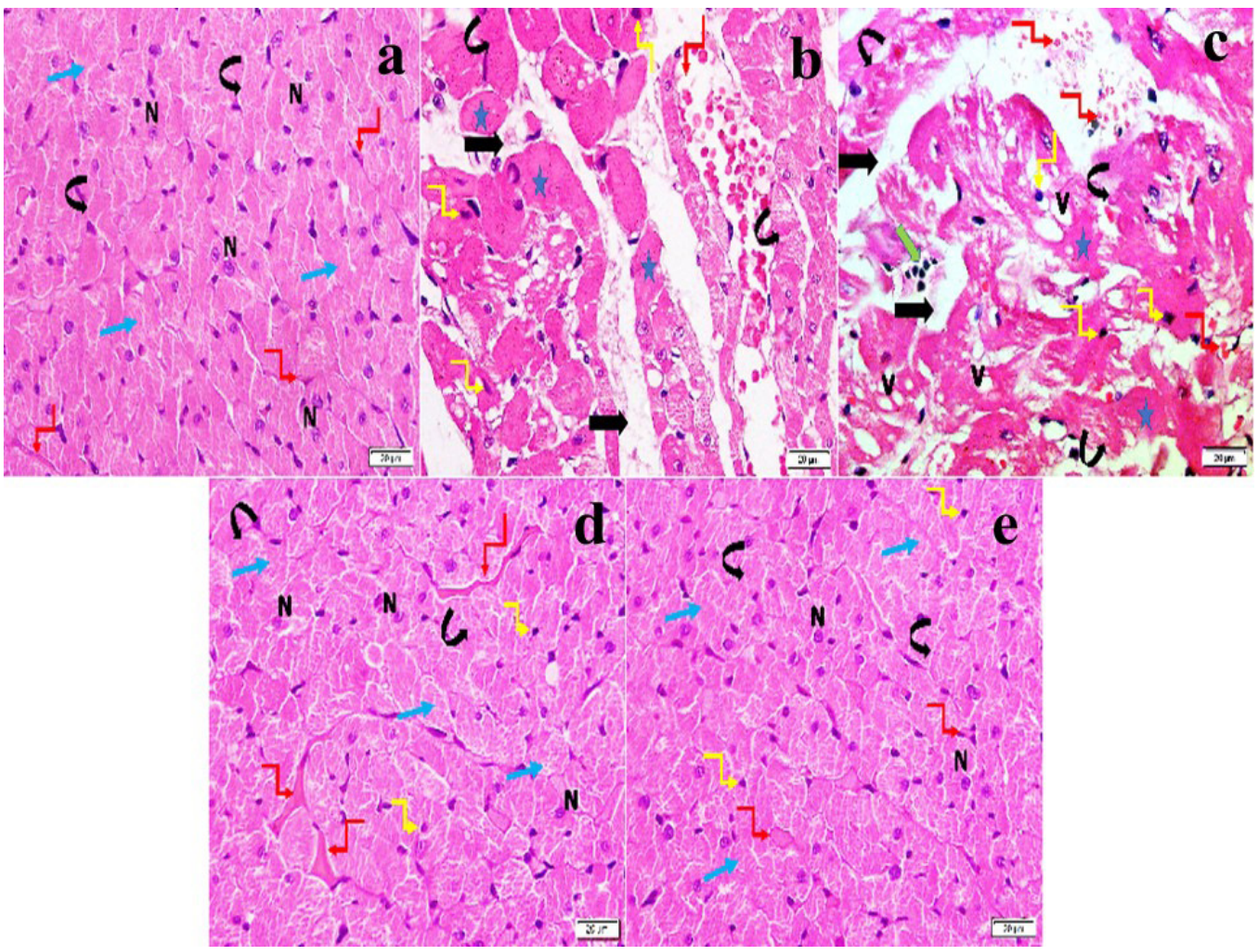


**Fig. 2:** Photomicrographs of transverse sections in the apex of the left ventricle (H&E, X400).

**a:** The control groups (I) show normal polyhydral muscle fibers exhibiting acidophilic sarcoplasm (blue arrows), central vesicular oval nuclei (Letter N) and clear sarcolemma (black curved arrows). Note the presence of small blood vessels (red kinked arrows) in between the muscle fibers.

**b:** cisplatin subgroup (II-a) shows widely separated muscle fibers (arrows) with focal areas of deeply acidophilic sarcoplasm (blue stars) and dissolved sarcolemma (black curved arrows). Some fibers show darkly stained pyknotic nuclei (yellow kinked arrows). Extravasated RBCs (red kinked arrow) are also seen.

**c:** Spontaneous recovery subgroup (II-b) reveals widely separated muscle fibers (black arrows) with darkly stained pyknotic nuclei (yellow kinked arrows) and dissolved sarcolemma (black curved arrows). Focal areas of the sarcoplasm appear deeply acidophilic (blue stars) and others appear vacuolated (Letter V). Extravasated RBCs (red kinked arrows) and many cellular infiltration (green arrow) are also seen.

**d:** EMPA-treated subgroup (II-c) shows many well-organized muscle fibers with acidophilic cytoplasm (blue arrows) and clear sarcolemma in most of fibers with dissolved sarcolemma (black curved arrows) in few fibers. Some fibers have central pale vesicular nuclei (Letter N) and others have dark pyknotic nuclei (yellow kinked arrows). Notice the presence of congested blood vessels (kinked red arrows).

**e:** PRP-treated subgroup (II-d) shows nearly normal cardiac muscle fibers with vesicular central oval nuclei (Letter N), acidophilic sarcoplasm (blue arrows) and clear sarcolemma (black curved arrows). Few fibers have dark pyknotic nuclei (yellow kinked arrows). Small blood vessels (red kinked arrows) in between the muscle fibers are also seen.

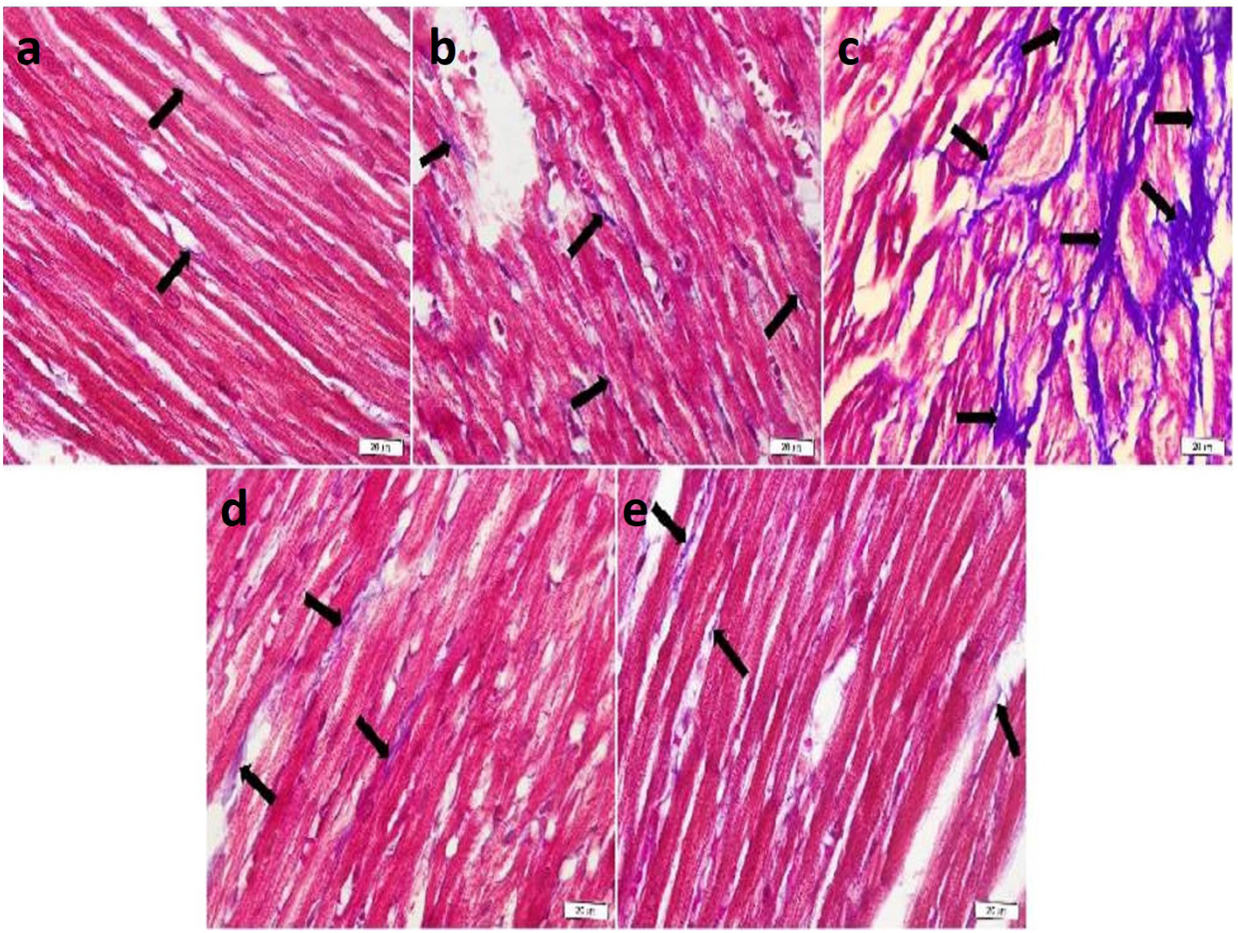


**Fig. 3:** Photomicrographs of longitudinal sections in the apex of the left ventricle (Mallory's trichrome, X400).

**a:** The control groups (I) exhibit fine collagen fibers (arrows) between the cardiac muscle fibers.

**b:** cisplatin subgroup (II-a) shows increased amount of collagen fibers (arrows) between the muscle fibers compared to the control.

**c:** Spontaneous recovery subgroup (II-b) reveals marked deposition of irregular collagen fibers (arrows) between the muscle fibers.

**d:** EMPA-treated subgroup (II-c) shows reduced amount of collagen fibers (arrows) between the muscle fibers compared to subgroups II-a and II-b.

**e:** PRP-treated subgroup (II-d) shows minimal amount of collagen fibers (arrows) between the muscle fibers.

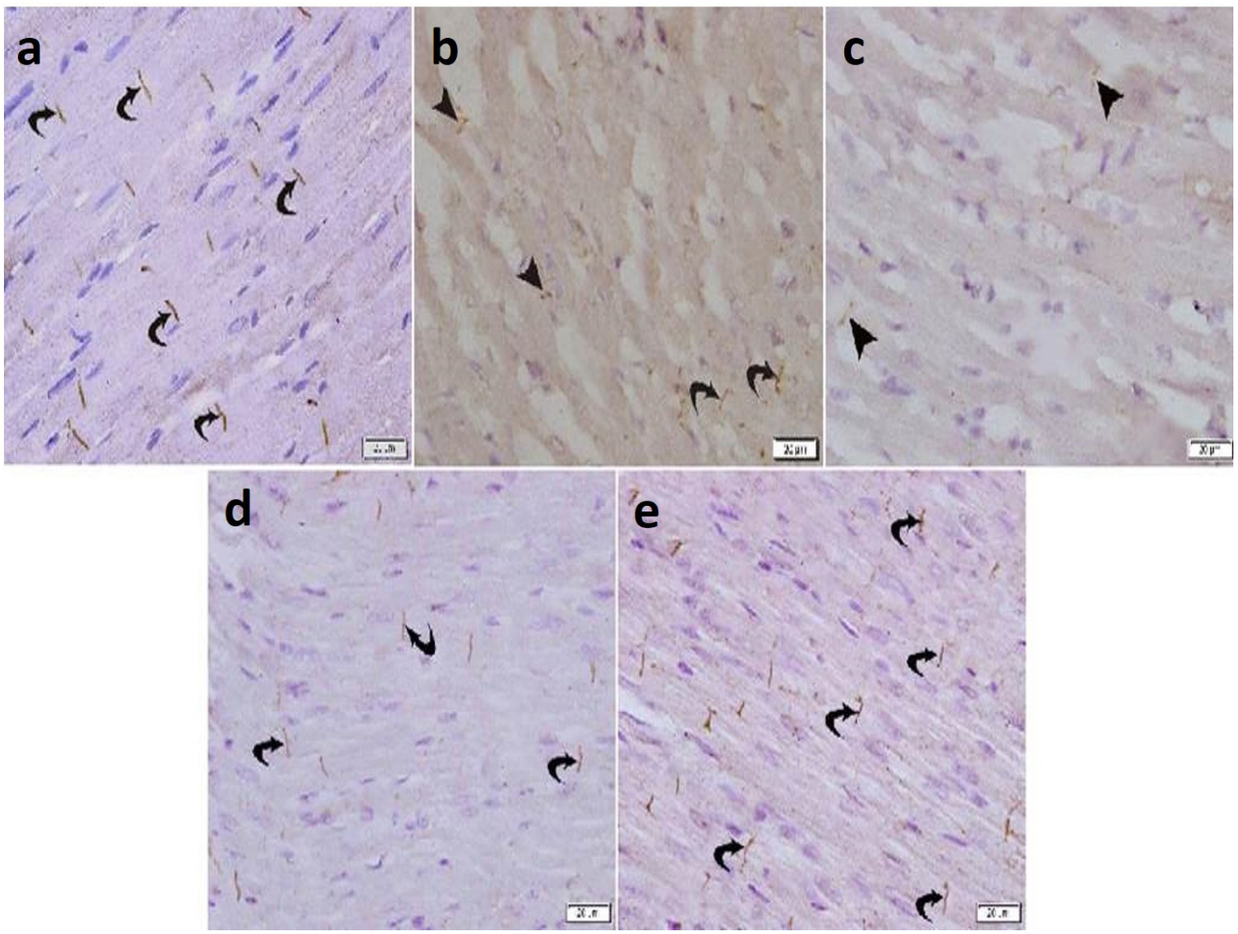


**Fig. 4:** Photomicrographs of longitudinal sections in the apex of the left ventricle (Cx43 immunostaining, X400).

**a:** The control groups (I) show multiple positive intercalated discs (curved arrows) between the cardiac muscle cells.

**b:** cisplatin subgroup (II-a) exhibits few positive intercalated discs (curved arrows) with many fibers showed disrupted discs (arrowheads).

**c:** Spontaneous recovery subgroup (II-b) shows few positive disrupted intercalated discs (arrowheads).

**d:** EMPA-treated subgroup (II-c) shows some positive intercalated discs (curved arrows).

**e:** PRP-treated subgroup (II-d) shows multiple positive intercalated discs (curved arrows).

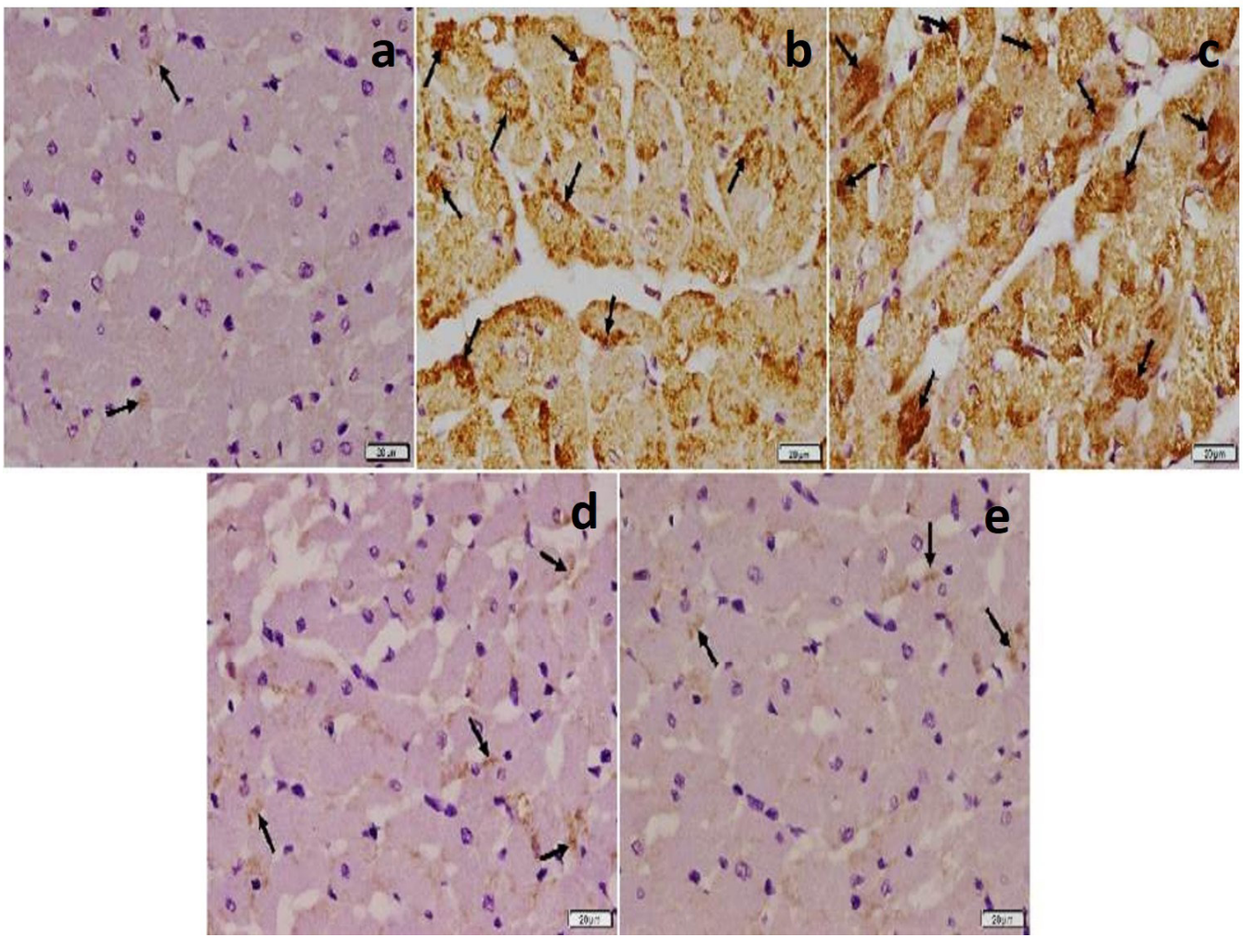


**Fig. 5:** Photomicrographs of transverse sections in the apex of the left ventricle (Caspase 3 immunostaining, X400).

**a:** The control groups (I) show faint immunostaining of the sarcoplasm (arrows).

**b:** cisplatin subgroup (II-a) shows strong sacroplasmic immunostaining in many fibers (arrows).

**c:** Spontaneous recovery subgroup (II-b) reveals strong sacroplasmic immunostaining in most of fibers (arrows).

**d:** EMPA-treated subgroup (II-c) exhibits mild sarcoplasmic immunostaining in some fibers (arrows).

**e:** PRP-treated subgroup (II-d) shows minimal sarcoplasmic immunostaining in few fibers (arrows).

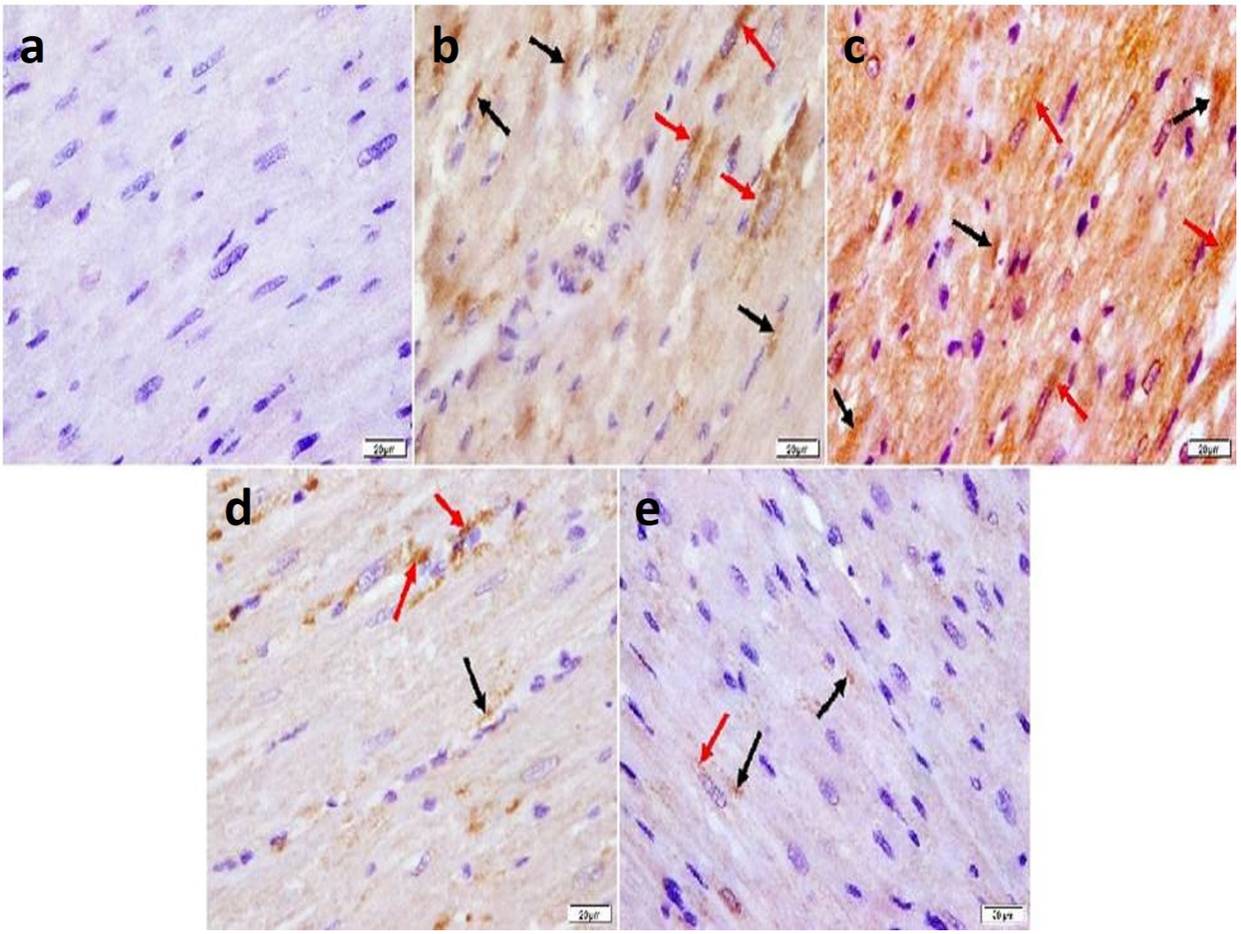


**Fig. 6:** Photomicrographs of longitudinal sections in the apex of the left ventricle (Fibronectin immunostaining, X400).

**a:** The control groups (I) show negative immunoreactivity.

**b:** cisplatin subgroup (II-a) exhibits positive immunoreactivity in the sarcoplasm of many cardiomyocytes (red arrows) and focal areas of ECM (black arrows).

**c:** Spontaneous recovery subgroup (II-b) shows strong positive and wide distributed immunoreactivity in both cardiomyocytes (red arrows) and ECM (black arrows).

**d:** EMPA-treated subgroup (II-c) shows mild to moderate immunoreactivity in few cardiomyocytes (red arrows) and limited areas of ECM (black arrow).

**e:** PRP-treated subgroup (II-d) reveals faint immunoreactivity in both cardiomyocytes (red arrow) and ECM (black arrows).

**Table 1:** Mean values ± SD of serum CK-MB, cTnI, SOD & MDA levels in control and experimental subgroups

| Groups | CK-MB (U/L) | cTnI (pg/mL) | SOD (µ/mg) | MDA (nmol/mg) |
|---|---|---|---|---|
| Control group (I) | 143.14±0.78 | 7.50± 0.20 | 2.73±0.32 | 0.81 ± 0.23 |
| Cisplatin subgroup (II-a) | 398.17±6.68[a] | 298.53±0.11[a] | 0.54±0.28[c] | 20.40 ± 0.69[a] |
| Spontaneous recovery subgroup (II-b) | 412.21±4.73[a] | 330.42±0.83[a] | 0.46±0.92[c] | 20.08 ± 0.97[a] |
| EMPA-treated subgroup (II-c) | 239.68±6.97[b] | 132.27±0.26[b] | 1.94±0.36[d] | 3.13 ± 0.39[b] |
| PRP-treated subgroup (II-d) | 151.12±4.51 | 9.78 ± 0.76 | 2.42±0.12 | 1.02± 0.22 |

**a** significant increase compared to group I and subgroups II-c & II-d.
**b** significant increase compared to group I & subgroup II-d.
**c** significant decrease compared to group I and subgroups II-c & II-d.
**d** significant decrease compared to group I & subgroup II-d.

**Table 2:** Mean values ± SD of area% of collagen fibers, area % of connexin 43, area % of caspase 3 & area % of fibronectin in control and experimental subgroups

| Groups | Area % of Collagen fibers | Area % of connexin 43 | Area % of caspase 3 | Area % of fibronectin |
|---|---|---|---|---|
| Control group (I) | 1.24±0.56 | 5.39±1.67 | 4.0 ± 0.81 | 0.00 ± 0.00 |
| Cisplatin subgroup (II-a) | 18.21±1.08[a] | 1.65±0.37[c] | 37.60 ± 7.22[a] | 6.92 ±0.64[a] |
| Spontaneous recovery subgroup (II-b) | 42.11±4.63[a#] | 1.01±0.92[c*] | 54.60 ± 6.31[a#] | 17.08 ± 1.07[a#] |
| EMPA-treated subgroup (II-c) | 5.27±1.41[b] | 2.89±0.74[d] | 18.03±2.51[b] | 3.44 ±0.93[b] |
| PRP-treated subgroup (II-d) | 2.06±1.36 | 4.80±1.04 | 06.20 ± 1.62 | 1.04± 0.32[e] |

**a** significant increase compared to group I and subgroups II-c & II-d.
**b** significant increase compared to group I & subgroup II-d.
**c** significant decrease compared to group I and subgroups II-c & II-d.
**d** significant decrease compared to group I & subgroup II-d.
**e** significant increase compared to group I.
**#** significant increase compared to subgroup II-a.
***** significant decrease compared to subgroup II-a.

## DISCUSSION

Cisplatin is a widely used drug in cancer chemotherapy. However, there are serious side-effects as cardiotoxicity has been reported with cisplatin in several studies[12]. The development of cardiac disorders during cisplatin therapy is a serious problem and the main question in this issue is, what is the best preventive & therapeutic strategy[21]. This study was conducted to assess and compare the potential therapeutic effect of EMPA and PRP on cisplatin induced cardiotoxicity in adult male albino rats.

In the current study, cisplatin cardiotoxicity was proved either after 1 or 4 weeks of cisplatin administration (subgroups II-a & II-b respectively) by biochemical markers which showed a significant increase in CK-MB, cTnI & MDA and a significant decrease in SOD serum levels as compared to the other subgroups. The adverse effects of cisplatin treatment on the cardiac muscle have been explained by several mechanisms which initiates peroxidation of membrane bound proteins and polyunsaturated fats (PUFAs) leading to cardiomyocyte damage by impairing oxidant-antioxidant balance[22].

This was in accordance with Khadrawy *et al*[23] who mentioned that cisplatin can generate ROS, such as hydroxyl radical and superoxide anion. These ROS are linked to an increase in lipid peroxidation resulting in creatinine kinase leakage from cardiac myocyte membranes.

Abdellatief *et al*[24] also reported that cisplatin increases the ROS production through NADPH oxidase and decreases some antioxidant enzymes, such as SOD, which could be referred to the interaction of excessive ROS with enzyme molecules, leading to partial inactivation and denaturation of these enzymes[25].

By LM examination, histopathological findings were observed after cisplatin administration (subgroups II-a & II-b) in the form of degeneration, discontinuation, and wide separation of cardiac muscle fibers with focal areas of either deeply acidophilic sarcoplasm or vacuolation with loss of striations. Darkly stained pyknotic nuclei, Blood extravasation and many cellular infiltrations were also observed. These findings can be explained by the excessive production of ROS that causes cardiotoxicity by altering the structures, integrity and functions of cells resulting in cardiac enzymes leakage that cause further myocardial damage[26]. Saleh *et al*[27] reported that ROS can induce overexpression of pro-inflammatory cytokines, that mediate inflammation and immune response. In addition, infiltration and neutrophils activation at the site of inflammation can result in damage by physical occlusion of the micro vessels, affecting the endothelial lining[25].

Regarding the deep acidophilic cytoplasm and pyknotic nuclei observed in our study, were explained by El-Sawalhi and Ahmed[28] who observed that following cisplatin administration, there is significant nuclear DNA fragmentation in cardiac tissues, which suggests apoptotic cell death. Apoptosis was further confirmed by caspase-3 immunostaining results of subgroups II-a & II-b which showed a significant increase in the mean area% of caspase-3 immunopositive cells as compared to the other experimental subgroups.

Cardiac muscle fibrosis in this study was confirmed by Mallory Trichrome and fibronectin immunostaining results which showed a significant increase in mean area % of collagen fibers and fibronectin after cisplatin administration (subgroups II-a & II-b) in comparison with the other subgroups. This was consistent with Shredah[29] who explained these results by lipid peroxidation which triggers an inflammatory response with overproduction of fibrogenic cytokines, which induce fibrosis. Moreover, Hazzaa *et al*[30] added that, macrophages may be responsible for increasing fibronectin and platelet derived growth factor; both of which stimulate fibroblasts division.

Gap junctions are clustered channels consisting of two hemichannels, each made up of six connexin proteins (Cx). Cx43 is the primary one expressed in cardiac muscle cells[31]. The present results showed a significant decrease in mean area % of Cx43 immunoreactivity after cisplatin administration in subgroups II-a & II-b as compared to the other subgroups. In accordance, Salameh and Dhein[32] clarified that cisplatin reduced gap junction intercellular communication through reduction in Cx43 expression.

EMPA is an antidiabetic drug that is an SGLT2 inhibitor and has recently been proven to significantly decrease cardiovascular mortality rate and HF hospitalization[33]. In this study, the EMPA-treated subgroup (II-c) showed significant decrease in CK-MB, cTnI & MDA and significant increase in SOD levels as compared to cisplatin administration subgroups (II-a & II-b). This agreed with Li *et al*[34] who mentioned that EMPA has cardioprotective effect by inhibiting of NADPH oxidases induced oxidative stress in diabetic cardiomyopathy. Said and Abdallah,[35] also reported the antioxidant effect of EMPA in diabetic nephropathy by increasing SOD level and decreasing MDA level.

By microscopic examination, EMPA-treated subgroup (II-c) showed many well-organized muscle fibers with transverse striations, intact sarcolemma and intercalated discs. Some fibers have central pale vesicular nuclei and others have dark pyknotic nuclei. Few cellular infiltration and congested blood vessels were also detected. In addition, there were a significant decrease in the mean area % of collagen fibers, caspase 3 and fibronectin immunoreactivity and a significant increase in the mean area % of Cx43 immunoreactivity in EMPA-treated subgroup (II-c) as compared to cisplatin subgroups (II-a & II-b). This improvement in the cardiac muscle structure in EMPA-treated subgroup may be referred to a combined modulation of caspase 3 and lipid peroxidation resulting in reduction of ROS production and cardiac myocytes apoptosis[36]. Bertero *et al*[37] also reported that EMPA maintained cardiovascular endothelial cell function by suppressing mitochondrial ROS production, anti-apoptotic, anti-inflammatory and antioxidant effects. Asensio Lopez *et al*[38] added that EMPA treatment reduced pro-fibrotic and pro-hypertrophic molecules and interstitial fibrosis. Recent studies suggested cardioprotection of SGLT2 inhibitors through induction of autophagy[39].

We observed that the attenuation effect of PRP on cisplatin-induced cardiotoxicity was more obvious than the attenuation effect of EMPA as PRP-treated subgroup (II-d) showed a significant decrease in CK-MB, cTnI & MDA and significant increase in SOD levels as compared to EMPA-treated subgroup (II-c). This was agreed with Moch. Rizal *et al*[40] who reported that PRP can increase the antioxidant enzymes biosynthesis, which repair lipid peroxidation induced damage. In addition, PRP can suppress oxidative stress by decreasing ROS production, lowering MDA level and increasing antioxidant defense.

At the microscopic level, PRP-treated subgroup (II-d) showed apparently normal cardiac muscle fibers separated by fine endomysium. Few fibers with dark pyknotic nuclei were also noticed. Thus, we observed that, PRP had a better effect on the cardiac muscle histological structure than EMPA as subgroup II-d showed a significant increase in the mean area % of Cx43 immunostaining and a significant decrease in the mean area% of collagen fibers, caspase 3 and fibronectin immunostaining as compared to EMPA-treated subgroup (II-c). This decrease in caspase 3 immunostaining confirmed the cardiac muscle plasticity which is the ability of cardiac muscle to replace necrotic or apoptotic cardiomyocytes after injury. This regeneration can be achieved by reprogramming non-cardiomyocytes into cardiomyocytes, the differentiation of pluripotent stem cells into cardiomyocytes, and the proliferation of pre-existing cardiomyocytes[41].

This significant improvement in cardiac muscle structure has been explained by Mishra *et al*[42] who referred the regenerative effect of PRP to its content of various cytokines and growth factors such as VEGF and insulin-like growth factor-1 (IGF-1) which can protect cardiac progenitor cells (CPC) from death and promote proliferation of adult cardiomyocytes. IGF-1 can also enhance the migration of mesenchymal stem cells from bone marrow to the site of myocardial injury. Furthermore, platelets have anti-inflammatory properties due to the inhibition of the nuclear factor-kB pathway, and they can circulate in an active state, forming complexes with other inflammatory and immune cells in many disorders[43]. Also, Sadeghinia *et al*[44] added that PRP enhances angiogenesis, proliferation and differentiation of stem cells via paracrine and autocrine pathways.

Concerning fibrosis, PRP showed greater antifibrotic effect which was clarified by Attia *et al*[43] who mentioned that PRP contains anti-fibrotic molecules, serum amyloid protein and hepatocyte growth factor which can suppress fibrosis and control macrophage function. In addition, Salem *et al*[45] proved that PRP has anti-apoptotic effect by reducing the mRNA level of caspase-3 and Bcl-2-associated death promoter (BAD) and increased the level of mRNA of Bcl-2 protein (anti-apoptotic regulator) which may provide a new approach to the treatment of various diseases.

## CONCLUSION

In conclusion, this study demonstrated that EMPA and PRP significantly improved cisplatin-induced cardiac damage by alleviating oxidative stress, suppressing inflammation and inhibiting apoptosis confirmed by serological, histological, immunohistochemical markers and morphometric studies The current findings demonstrated a more obvious improvement in response to PRP than EMPA suggesting its possible use as a therapeutic approach for attenuating toxin-induced cardiac damage in the future.

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## CONFLICT OF INTERESTS

There are no conflics of interest.

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**الملخص العربى**

# دراسة نسيجية وكيميائية مناعية مقارنة للتأثيرات العلاجية المحتملة للإمباغليفلوزين والبلازما الغنية بالصفائح الدموية ضد السمية القلبية المستحثة بالسيسبلاتين في الجرذان

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**الخلفية والأهداف:** سيسبلاتين هو دواء علاجي كيميائي فعال للغاية يستخدم لعلاج أنواع مختلفة من السرطان. ومع ذلك ، فإن السمية القلبية هي واحدة من أهم الآثار الضارة له. هدفت هذه الدراسة إلى التحقيق ومقارنة التأثيرات العلاجية المحتملة للإمباغليفلوزين والبلازما الغنية بالصفائح الدموية على السمية القلبية المستحدثة بالسيسبلاتين في ذكور الجرذان البيضاء البالغة.

**المواد والأساليب:** تم تصنيف ٤٨ ذكورًا من الجرذان البيضاء البالغة إلى: المجموعة الأولى (المجموعة الضابطة) والمجموعة الثانية (المجموعة التجريبية). تم حقن جميع الجرذان في المجموعة الثانية مرة واحدة بسيسبلاتين (١٠ مجم / كجم) داخل التجويف الصفاقي ثم بعد ٧ أيام تم تقسيمها إلى ؛المجموعة الفرعية سيسبلاتين (٢-أ ) ؛ تم التضحية بالجرذان في اليوم السابع ، تركت المجموعة الفرعية للانتعاش التلقائى (٢-ب) دون علاج لمدة ٣ أسابيع ، تلقت المجموعة الفرعية المعالجة بـ الامباغليفلوزين (٢-ج) الامباغليفلوزين يوميا عن طريق الفم (٢٠ مجم / كجم / يوم) لمدة ٣ أسابيع ، وتلقت المجموعة الفرعية المعالجة بالبلازما الغنية بالصفائح الدموية (٢-د) ٠,٥ مل / كجم من البلازما الغنية بالصفائح الدموية تحت الجلد مرتين أسبوعياً لمدة ٣ أسابيع. في نهاية التجربة ، تم جمع عينات الدم لقياس الكرياتين كيناز، التروبونين القلبي، السوبراوكسيد ديسميوتاز و مالون-داي-ألديهيد. تم الحصول على عينات عضلة القلب من أجل التحليل النسيجي والكيميائي المناعي والمورفومتري والإحصائي.

**النتائج:** حدث زيادة كبيرة في متوسط مستوى الكرياتين كيناز، التروبونين القلبي و مالون-داي-ألديهيد مرتبطة بانخفاض كبير في مستوى السوبراوكسيد ديسميوتاز في المجموعة الفرعية (٢-أ) والمجموعة الفرعية (٢-ب). بالإضافة إلى ذلك ، أظهرت المساحة المئوية لألياف الكولاجين وكاسباس ٣ والفيبرونيكتين زيادة ذات دلالة احصائية و أظهرت المساحة المئوية للكونيكسون انخفاضًا ذا دلالة احصائية في المجموعة الفرعية (٢-أ) والمجموعة الفرعية (٢-ب). بينما أظهرت المجموعة الفرعية (٢-ج) والمجموعة الفرعية (٢-د) تحسنًا ملحوظًا في العلامات البيوكيميائية والتغيرات النسيجية والهيستوكيميائية المناعية التي سببها السيسبلاتين مع تحسن أكثر وضوحًا في المجموعة الفرعية (٢-د).

**الاستنتاج:** اظهراعطاء الإمباغليفلوزين والبلازما الغنية بالصفائح الدموية تحسناً ملحوظا في تلف القلب الناجم عن السيسبلاتين عن طريق تخفيف الإجهاد التأكسدي، وقمع الالتهاب وتثبيط موت الخلايا المبرمج ؛ وقد كان التحسن أكثر وضوحا في الاستجابة للبلازما الغنية بالصفائح الدموية.