Kingdom of Saudi Arabia
Ministry of Higher Education
Qassim University
College of Pharmacy
Department of Pharmacology & Toxicology

Ameliorative Effect of Empagliflozin and Linagliptin on Cisplatin-Induced Nephrotoxicity and Cardiotoxicity

A Thesis submitted in partial fulfillment of the requirements for the Master's Degree in Pharmacology

By

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Recommendation of the Committee:
The Committee has approved this dissertation as a partial completion of the requirement for The master's degree in Pharmacology
Examination and Decision-Making Committee

Committee Members	Name	Academic Degree	Specialties Action	Signature
Supervisor	Prof. Maha Aldubayan	Professor	Pharmacology and Toxicology	
Internal Examiner	Prof. Vasudevan Mani	Professor	Pharmacology and Toxicology	
Internal Examiner	Prof. Ahmad Alhowail	Professor	Pharmacology and Toxicology	


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Abstract

Background: The therapeutic usage of cisplatin, an extremely efficient chemotherapeutic drug, was constrained by its adverse impact on various organs, including the heart and kidneys. Evidence showed that new antidiabetic medications improve cardiorenal function in non-diabetic and diabetic clients. This study aims to assess the ameliorative outcome of linagliptin and empagliflozin (each solely and in combination) on cisplatin-induced cardiorenal toxicity in non-diabetic rats.

Methods: Forty nine rats were indiscriminately distributed into seven equal groups: Control (saline 10 ml /kg), Cisplatin group (CP 20 mg/kg), Empagliflozin group (EMPA10 mg/kg), Linagliptin group (LINA 3 mg/kg) and treated groups of (CP + EMPA, CP+LINA, CP+EMPA+LINA). All groups were treated by oral route for 10 days, and CP (20 mg/kg single intraperitoneal dose) was administered to the treated groups. Samples of blood were collected from the retro-orbital plexus for evaluation of biochemical parameters such as Creatinine, blood urea nitrogen (BUN), creatine kinase (CK-MB), lactate dehydrogenase (LDH), and troponin I. Animals hearts and kidneys were extracted to evaluate the activity of malonaldehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX).

Results: The results showed a noteworthy rise in Creatinine, BUN, LDH, CK-MB, troponin I and MDA in the CP group in compared with the control. In contrast, treated groups (CP+LINA, CP+EMPA, CP+LINA+EMPA) exhibited a notable reduction in CRE, BUN, LDH, CK-MB, troponin I and MDA in comparison with CP group. Furthermore, SOD, GPX, and CAT levels were significantly decreased in CP group (in both cardiac and kidney tissues) in comparison with the control. While, the same parameters (SOD, GPX, and CAT) were considerably rise in the treated groups in comparison with CP group except that CP+LINA group revealed a non-notable increase in cardiac SOD and CAT.

Conclusion: This study revealed that empagliflozin and linagliptin (solely and in combination) improved cardiorenal function and oxidative stress biomarkers in rats treated with cisplatin, which may reflect their ameliorative effect on cardiorenal toxicity induced by cisplatin. Therefore, they could be promising approaches to mitigate cardiorenal damage associated with cisplatin treatment.

Keywords: Cisplatin; Empagliflozin; Linagliptin; oxidative stress biomarkers, Cardiorenal toxicity

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List of Abbreviation

AKI	Acute kidney injury
BCA	Bicinchoninic Acid Protein Assay
BUN	Blood urea nitrogen
CAT	Catalase
cAMP	cyclic adenosine monophosphate
CK-MB	Creatine kinase- myoglobin binding
CP	Cisplatin
CRE	Creatinine
DPP-4i	Dipeptidyl-peptidase-4 inhibitors
EMPA	Empagliflozin
ELISA	Enzyme-Linked Immunosorbent Assay
ER	endoplasmic reticulum
GFR	Glomerular filtration rate
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide-1
GLP-1RA	Glucagon-like peptide-1 receptor agonists
GPX	Glutathione peroxidase
HO-1	Heme oxygenase-1 expression
LDH	Lactate dehydrogenase
LINA	Linagliptin
MAPK	Mitogen-activated protein kinase
MDA	Malondialdehyde
MPTPs	Mitochondrial permeability transition pores
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NF-κB	nuclear factor kappa-B
NOX4	Nicotinamide Adenine Dinucleotide Phosphate Oxidase 4
PKA	protein kinase A

ROS	Reactive oxygen species
SOD	Superoxide dismutase
SGLT2	sodium-glucose cotransporter 2
SGLT2i	Sodium-glucose cotransporter-2 inhibitors
T2DM	type 2 diabetes mellitus
WHO	World Health Organization

CHAPTER I:
Introduction

1.1 Introduction

Cancer is responsible for approximately 459,000 deaths per year in the Eastern Mediterranean region (WHO, 2023) [1]. In Saudi Arabia, the incidence of cancer is anticipated to reach 60,429 by 2040. Cancer represents significant challenges not only in terms of its treatment but also in managing the side effects associated with therapeutic interventions [2]. Cisplatin (CP) is a platinum-based medicine classified as an alkylating agent within cytotoxic medications. It is prescribed for several cancer types, such as sarcomas, and certain carcinomas like small cell lung cancer, ovarian cancer, lymphomas, and germ cell tumors [3]. Despite the reliability and efficacy of platinum-based chemotherapeutic agents in cancer treatment, the adverse effects of cisplatin, including cardiotoxicity, nephrotoxicity, hepatotoxicity, and hypothyroidism, limit its application [4,5].

Nephrotoxicity with CP affects about 20-50% of individuals. This usually presents as acute kidney injury(AKI) within days of exposure and can lead to both immediate and prolonged health issues [6]. CP may induce cardiac damage by elevating specific cardiac biomarkers, including troponin I, creatine kinase (CK), and CK-MB [7]. CP-induced cardiotoxicity encompasses arrhythmias, atrial fibrillation, angina pectoris, congestive heart failure, myocarditis, and sudden cardiac death. [8]. These cardiotoxic events can restrict the chemotherapeutic dosage of CP and elevate the long-term incidence of cardiovascular disease.

CP acts through a non-cell cycle-specific mechanism, primarily by covalently attaching platinum to the purine bases, namely guanine and adenine. This attachment results in both intra-strand and inter-strand crosslinks, leading to breaks in the DNA strands [9]. Aggregation of CP in cardiac tissues and renal proximal tubules stimulates nuclear factor kappa-B (NF-κB) and the mitogen- activated protein kinase (MAPK), causing immune cells like macrophages and neutrophils to infiltrate and produce proinflammatory cytokines. This results in inflammation, cellular apoptosis, and tissue injury. Moreover, the induction of mitochondrial dysfunction, and mitochondrial permeability transition pores (MPTPs) opening enhances the generation of ROS, which permits pro-apoptotic factors to enter the cytosol, thereby stimulating endoplasmic reticulum (ER) stress and apoptotic cell death. The initiation of these pathogenic mechanisms leads to tissue destruction and eventually heart and renal failure [8] (Figure 1).

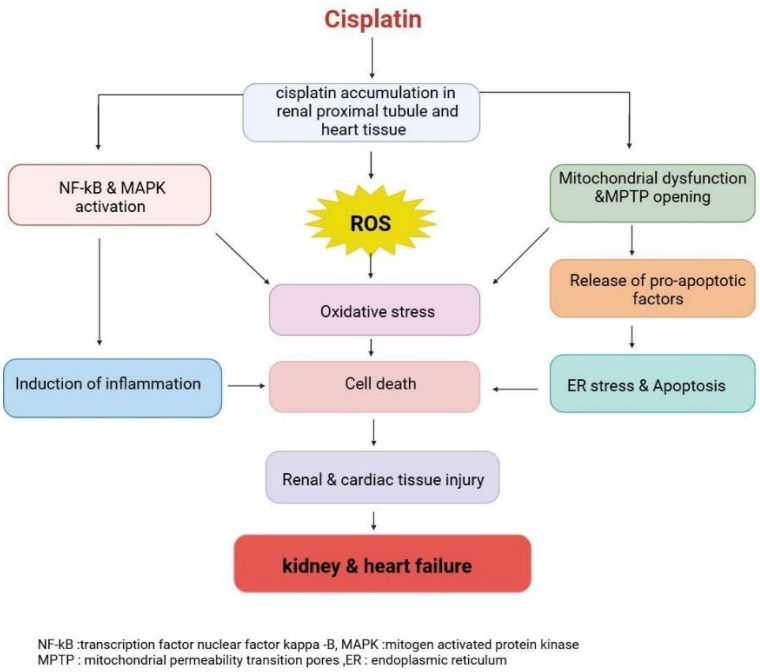


Figure 1: Schematic illustration of the pathophysiological occurrences in cisplatin-induced renal and cardiac toxicities

Adapted from: Dugbartey GJ, Peppone LJ, de Graaf IA. An integrative view of cisplatin-induced renal and cardiac toxicities: Molecular mechanisms, current treatment challenges, and potential protective measures. Toxicology. 2016; 371:58-66.

The introduction of innovative oral antidiabetic medications with unique modes of action has revolutionized type 2 diabetes mellitus (T2DM) treatment. These drugs comprise sodium-glucose cotransporter-2 inhibitors (SGLT2i), glucagon like peptide-1 receptor agonists (GLP-1RA), and dipeptidyl- peptidase-4 inhibitors (DPP-4i). These drugs ameliorate adverse renal and cardiac events, irrespective of their anti-hyperglycemic actions. [10-13].

In the kidney, over 90% of glucose reabsorption occurs through sodium-glucose cotransporter 2 (SGLT2) which is located in the proximal convoluted renal tubule. Consequently, inhibiting this transporter increases glucose and sodium excretion in the urine, thus lowering increased blood glucose levels, blood pressure, and body weight. [14,15]. SGLT2i has demonstrated significant cardiovascular and renal advantages in individuals with T2DM [16,17] . Moreover, the cardiorenal protective effect of SGLT2i was evident by several animal studies in nondiabetic chronic kidney disorders [18-20] .

Numerous hemodynamic and nonhemodynamic pathways are postulated to mediate SGLT2i's protective effects on the kidney. The activation of tubuloglomerular feedback is believed to be the

primary cause. The SGLT2i mechanism in the proximal convoluted tubule increases sodium concentrations at the macula densa. This mostly induces vasoconstriction of the afferent arterioles via adenosine-mediated signaling pathways, resulting in decreased intraglomerular pressure and less hyperfiltration-related injury [21] (Figure 2).

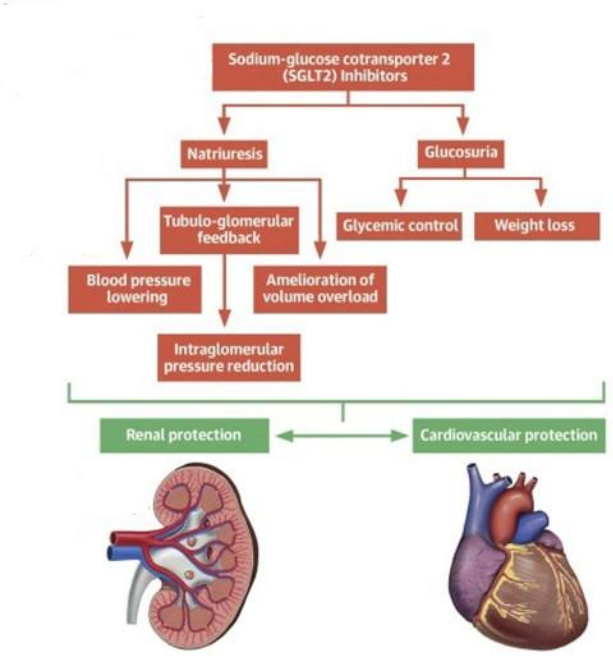


Figure 2: Proposed cardiorenal protection mechanisms with SGLT2 inhibitors
Adapted from: Vergara A, Jacobs-Cachá C, Soler MJ. Sodium-glucose cotransporter inhibitors: beyond glycaemic control. *Clinical kidney journal*. 2019;12(3):322-325.

Various metabolic and hemodynamic processes that enhance myocardial performance, either directly or indirectly may mediate the protective effect of SGLT2i on the heart. [22,23]. SGLT2i diminish cardiac afterload by decreasing arterial pressure without elevating heart rate and have demonstrated a reduction in arterial stiffness [24,25]. Moreover, these drugs elevate glucagon levels resulting in favorable inotropic and chronotropic actions in myocardial cells [26]. Inhibition of SGLT2 enhances cardiac ATP synthesis by elevating the rates of cardiac oxidation of glucose and fatty acids, as well as increasing the supply and oxidation of ketones by the heart [27]. Augmented renal ketogenesis may contribute to elevated hematocrit levels in SGLT2 inhibition, enhancing oxygen transport to vascular regions prone to hypoxia, such as the renal medulla and heart [28].

DPP-4 inhibitors was established and authorized for the management of T2DM. Their principal mechanism involves suppression of incretin degradation, specifically GLP-1. Food consumption stimulates release of incretins by the small intestine, and GLP-1 promotes insulin secretion from the pancreas. Moreover , GLP-1 also exerts direct effects on the heart, blood vessels, and kidneys, mostly

via the GLP1 receptor [29]. DDP-4i provides renoprotective and cardioprotective benefits through GLP1 dependent and independent pathways (Figure 3) .

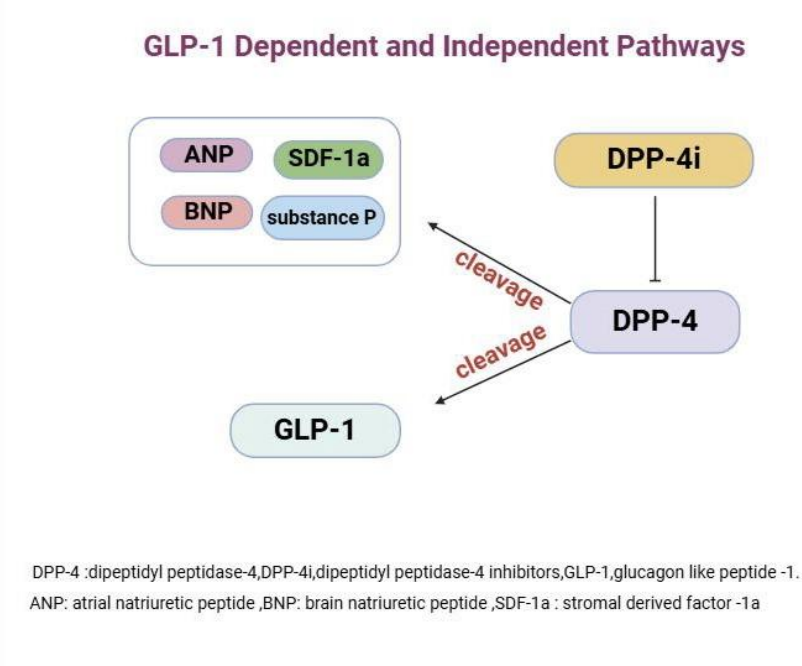


Figure 3: Mechanism effect of DDP-4 Inhibitors on kidney and heart

GLP-1RA induces diuresis and natriuresis through GLP-1 receptors[30]. inhibition of DPP-4 enzyme activity by DPP-4i results in elevation of intrinsic GLP-1 levels, which in turn diminishes ROS formation by reducing nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase activity in several organs [31,32]. GLP-1 enhances adenylyl cyclase activity, resulting in elevated levels of cyclic adenosine monophosphate (cAMP), which promotes protein kinase A (PKA) activity and upregulates the expression of heme oxygenase-1 (HO-1). HO-1 is a stress-induced protein that prevents diverse cells from oxidative damage [33,34] .

The DPP4 inhibition influences not only GLP-1 levels but also the concentrations of other peptides that may have cardioprotective effects, including ANP, BNP, SDF-1α, and substance P [35]. Atrial natriuretic peptide (ANP) which is formed in the atria and brain- natriuretic peptide (BNP) which is produced by the cardiac ventricles operates both locally and systemically to produce diuresis, natriuresis, vasodilation, and the suppression of renin and aldosterone secretion [36]. Stromal cell-derived factor 1(SDF-1α) is a substrate of the DPP4 enzyme, and the inhibition of DPP4 protects the effects of SDF-1α while facilitating heart recovery following ischemia/reperfusion injury. [37], acute myocardial

infarction [38], or stroke [39] Consequently, as a regulator of these peptides, DPP-4 may influence blood pressure, natriuresis, angiogenesis, and renal tissue healing [40]. Substance P is an additional DPP4 substrate that has potentially favorable effects on vascular function by increasing nitric oxide (NO) concentration and anticoagulation [41].

As numerous previous investigations indicate that CP produces ROS, which compromise the cell's antioxidant defense mechanism, leading to oxidative stress and exacerbating damage, ultimately resulting in renal and cardiac failure [42,43]. This study aims to examine the ameliorative effects of EMPA, LINA, and the combination of EMPA and LINA on cardiorenal toxicity caused by CP.

1.2 Research Hypothesis and Aims

Hypothesis:

CP-induced nephrotoxicity and cardiotoxicity through multiple mechanisms, including inducing inflammation, oxidative stress, and apoptosis. This study expected that EMPA and LINA would improve kidney and cardiac function through their involvement in reducing inflammation and oxidative stress.

Aims:

Aim 1: To explore the protective effects of EMPA against CP-induced cardiac and kidney injury.

Aim 2: To explore the protective effects of LINA against CP-induced cardiac and kidney injury.

Aim 3: To evaluate whether the combination of EMPA and LINA act synergistically to modulate renal and cardiovascular functions after CP administration.

Aim 4: To determine the molecular mechanism of CP on nephrotoxicity and cardiotoxicity.

CHAPTER II:

Materials and Methods

2. Materials and Methods

2.1 Drugs

CP (1 mg/ml) was purchased from EBEWE Pharma Ges.m.b.H. Nfg.KG, Austria. EMPA (10 mg) and LINA (5 mg) were purchased from Boehringer Ingelheim Pharmaceuticals, Germany.

2.2 Animals

Forty-nine male rats (200 - 250 g) were acquired from the animal housing at the College of Pharmacy, Qassim University. Rats were kept in cages containing five individuals each, maintained at a temperature of 25 ± 2 °C with access to water and food. Rats were acclimated for one week prior to the commencement of experimental procedures. The Institutional Animal Care and Use Committee approved the experimental procedure at the Deanship for Scientific Research at Qassim University (reference number 23-67-07), and the ethical rules of Qassim University were adhered to during the experiment.

2.3 Experimental Protocol and Treatment

Rats were indiscriminately allocated into seven groups, each consisting of seven rats. (N =7/group) as follows: The Control: received water orally and saline intraperitoneally (1 ml /kg) daily for 10 days. The second (CP) group received CP (20 mg/kg) a single dose intraperitoneally [44] . The third group (EMPA) received EMPA orally (10 mg/kg) for 10 days [45]. The fourth group (CP+EMPA) was administered with CP intraperitoneally (20 mg/kg) as a single dose intraperitoneally and EMPA orally (10mg/kg) for 10 days. The fifth group (LINA) received LINA (3 mg/kg) orally for 10 days [46]. The sixth group (CP + LINA) received CP (20 mg/kg) as a single dose intraperitoneally and LINA orally (3 mg/kg) for 10 days. The seventh group (CP + EMPA + LINA) administered a CP (20 mg/kg) single dose intraperitoneally, EMPA (10mg /kg) and LINA (3 mg/kg) by oral gavage for 10 days. The experimental design is described in Figure 4.

Upon completion of the experiment, blood samples were collected for the assessment of serum renal biomarkers (BUN, Creatinine) and cardiac biomarkers (CK-MB, LDH, and troponin I) utilizing the ELISA method. The rats were euthanized to extract the kidneys and heart for the assessment of oxidative stress indicators (SOD, GPX, CAT, and MDA) using a rat ELISA kit.

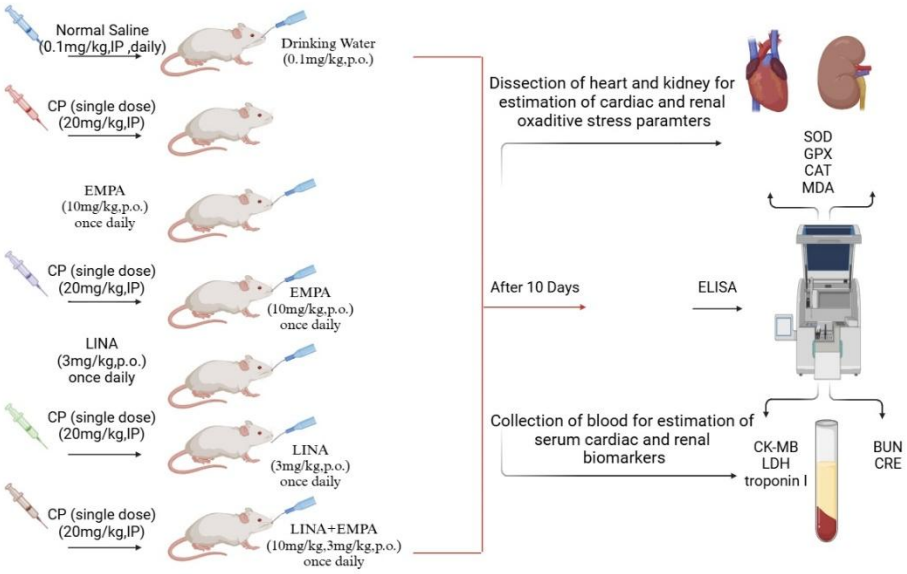


Figure 4: Experimental Protocol and Treatment Schedule

2.4 Blood collection and plasma separation

At the end of the experiment, the rats blood samples were collected in the heparin-coated tube by retro-orbital puncture, and the plasma was separated by centrifuging at 4000 rpm for 10 min. Blood plasma was transferred to new centrifuge tubes and stored at − 80 ◦C for further assay of renal function tests(BUN and creatinine), and serum cardiac enzymes (CK-MB, LDH, and troponin I)by a commercially available rat ELISA kit [47] (Figure 5).

2.5 Preparation of heart and kidney tissue homogenates

The rat's heart and kidneys were extracted. A 10% tissue homogenate was prepared in 0.1 M potassium phosphate buffer, pH 7.4. The homogenate was centrifuged at 1000 × g for 15 min at 4 °C. The clear supernatant was used for the estimation of oxidative stress markers (SOD, GPX, CAT, and MDA) utilizing a commercially available rat ELISA kit. [48] (Figure 6).

2.6 Bicinchoninic Acid Protein Assay (BCA)

The BCA assay standard curve and protein quantification were performed according to the method established by Smith et al. (1985). 200 µl of the standard working reagent, comprising 100 vol of BCA solution and 2 vol of 4% (w/v) copper (II) sulfate pentahydrate, was added to 25.0 µl samples containing 0.1-1.2 mg of protein standard solution . The plates were incubated at 37°C for 30 minutes, and absorbance was measured using a microplate reader [49] .

2.7 Enzyme-Linked Immunosorbent Assay (ELISA)

The previously produced protein samples were quantified using the BCA assay (Pierce, Waltham, MA, USA), and the levels of SOD, GPX, CAT, and MDA were evaluated with ELISA kits (ABclonal Technology, Woburn, MA, USA). The absorbance in each well was measured by an ELx800 Absorbance Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA) (60,61). The evaluation of serum renal biomarkers (BUN, Creatinine) and cardiac biomarkers (CK-MB, LDH, and troponin I) levels was obtained via an automated analyzer [50].

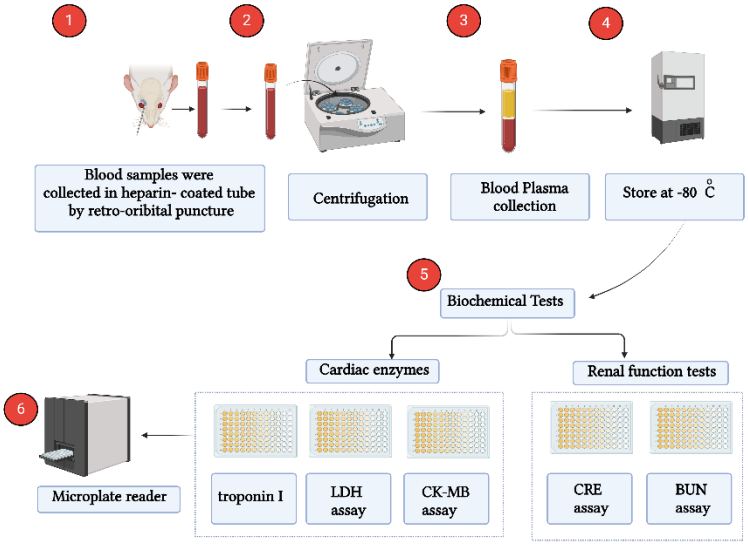


Figure 5 :Schematic diagram of the sample preparation(Blood) procedure proteomics analysis

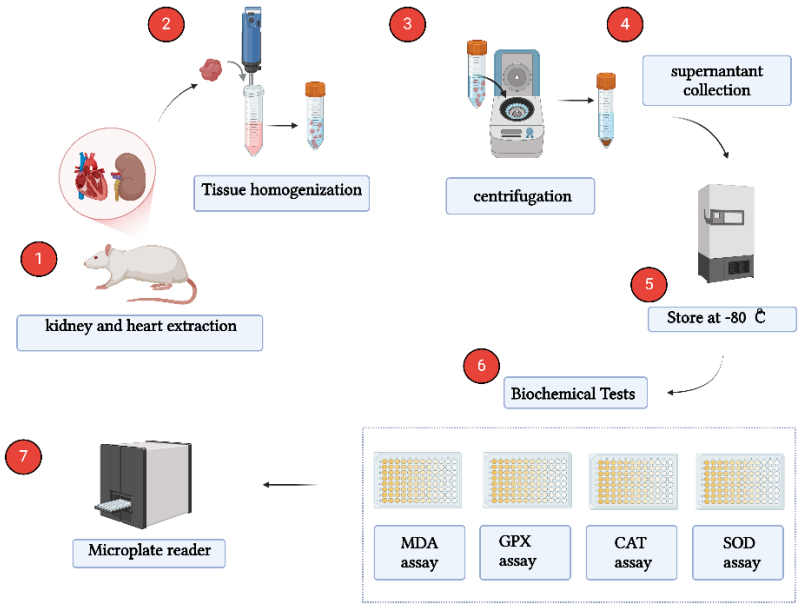


Figure 6 :Schematic diagram of the sample preparation(Tissue homogenization) procedure proteomics analysis

2.8 Statistical analysis

The results were analyzed utilizing GraphPad Prism 9 software. Oxidative stress biomarkers (SOD, GPX, CAT, and MDA) and renal and cardiac functions were evaluated using one-way analysis of variance (ANOVA) followed by Tukey-Kramer for multiple comparisons A p-value of less than 0.05 was deemed statistically significant.

CHAPTER III:
Results

3. RESULTS

3.1 Effect of LINA, EMPA alone, and in combination with CP on kidney parameters

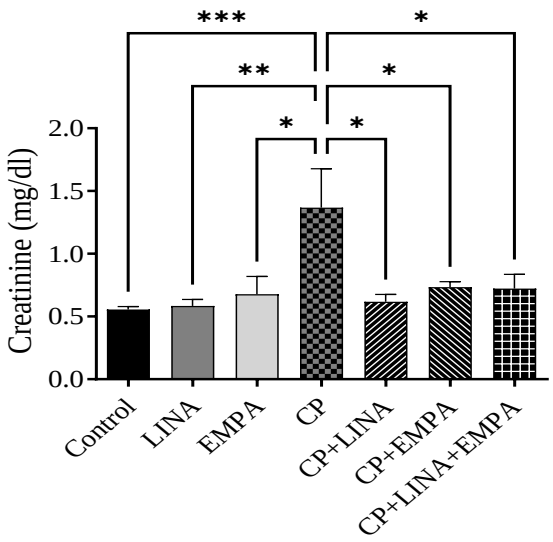


Figure 7(A)

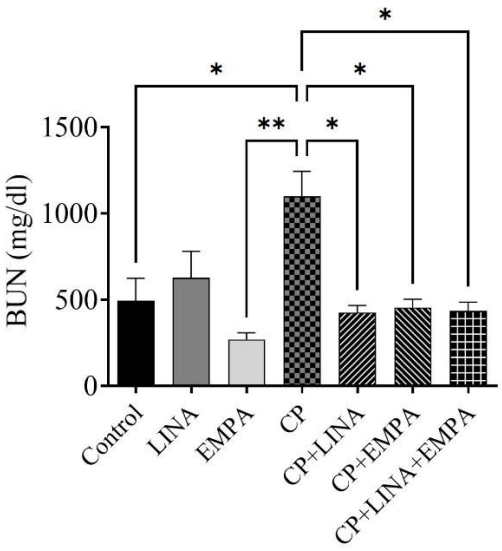


Figure 7(B)

Figure 7 : The effect of LINA, EMPA alone and in combination with CP on kidney parameters

(A) The effect of LINA, EMPA alone, and in combination with CP on Creatinine levels (B) The effect of LINA, EMPA alone, and in combination with CP on BUN levels. Note: LINA: Linagliptin; EMPA: Empagliflozin; CP: Cisplatin; BUN : Blood Urea Nitrogen N= 7; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to the control /CP group.

(Figure 7A) shows a noteworthy rise in Creatinine levels ($p < 0.001$) in the CP group compared to the control. In contrast, a considerable reduction in Creatinine levels was detected in treated groups (CP+LINA, CP+EMPA, and CP+LINA+EMPA) compared to the CP group ($p < 0.05$). Moreover, BUN levels significantly increased in the CP group compared to the control ($p < 0.05$). In addition, it was significantly reduced in the treated groups in comparison with the CP group ($p < 0.05$) (Figure 7B).

3.2 Effect of LINA, EMPA alone, and in combination with CP on cardiac parameters

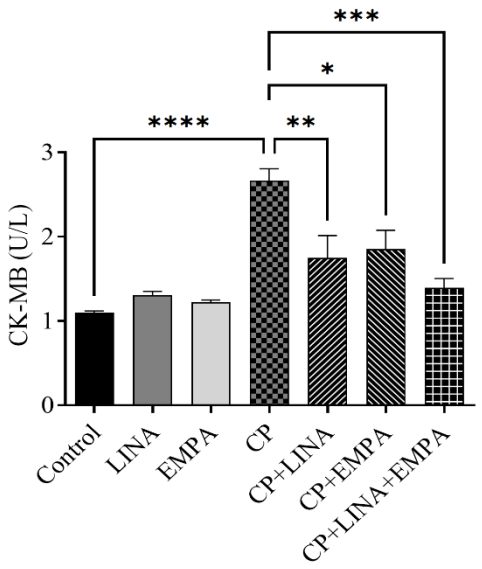


Figure 8(A)

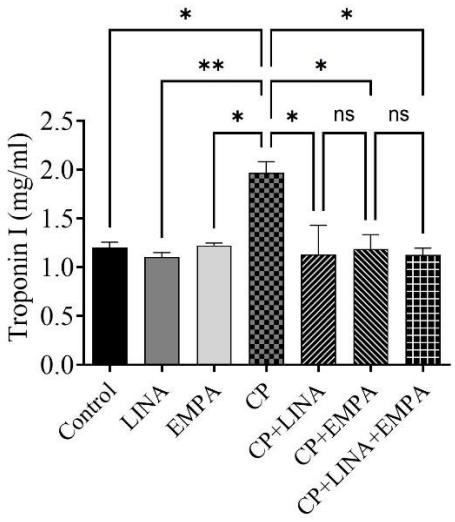


Figure 8(B)

Figure8 : Effect of LINA , EMPA alone and in combination with CP on cardiac parameters .

(A) The effect of LINA, EMPA alone and in combination with CP on troponin I levels ,(B) The effect of LINA, EMPA alone and in combination with CP on CK-MB levels .

Note: LINA: Linagliptin; EMPA: Empagliflozin; CP: Cisplatin; CK-MB: Creatine kinase; N= 7; *p < 0.05, **p < 0.01, ***P < 0.001 and ****P<0.0001 compared to the control /CP group .

Cardiac biomarkers evaluation shows that cisplatin considerably increased CK-MB in the CP group in comparison with control (P<0.0001), while the treated groups (CP+LINA, CP+EMPA, CP+LINA+EMPA) exhibited a significant reduction in CK-MB compared to CP group (P<0.01, P<0.05, P<0.001 respectively) (Figure 8A). Moreover, troponin I level showed a significant rise in the CP group compared to the control (P<0.05) and a considerable decrease in the treated groups compared to the CP group(P<0.05) (Figure 8B).

3.3 Effect of LINA, EMPA alone, and in combination with CP on Lactate dehydrogenase

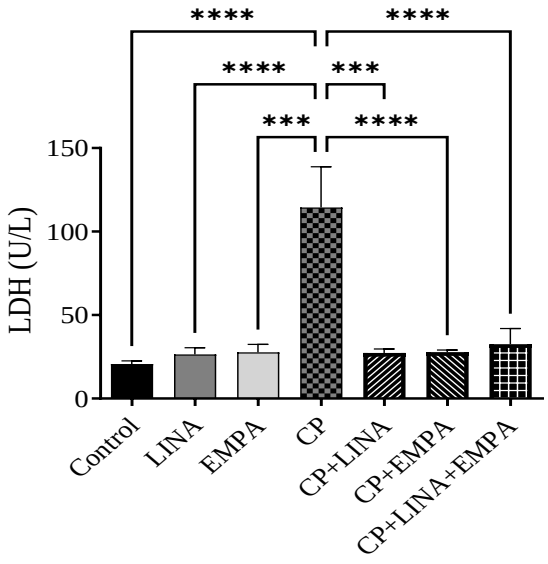
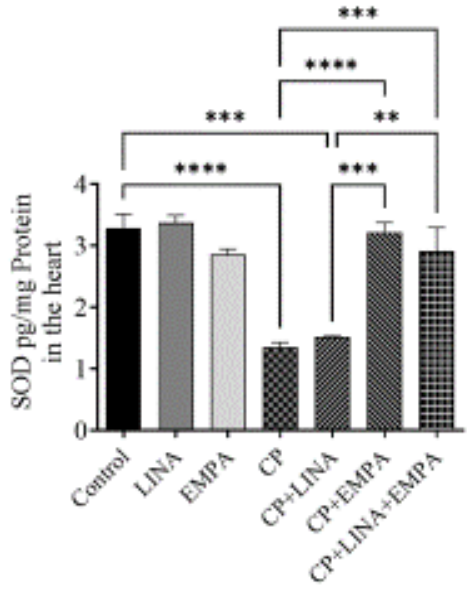


Figure 9: Effect of LINA , EMPA alone and in combination with CP on LDH levels .
Note: LINA: Linagliptin; EMPA: Empagliflozin; CP: Cisplatin; LDH: Lactate dehydrogenase; N= 7; ***p < 0.001 and ****p<0.0001 compared to the control /CP group.

Figure 9 shows a noteworthy rise in LDH (p <0 0001) in the CP group compared to the control. In contrast, a considerable reduction in LDH was detected in treated groups (CP+LINA, CP+EMPA, and CP+LINA+EMPA) compared to the CP group (P <0.0001, P<0.001, P<0.0001 respectively).

3.4 Effect of LINA, EMPA alone, and in combination with CP on oxidative stress parameter (Superoxide dismutase) in heart and kidney



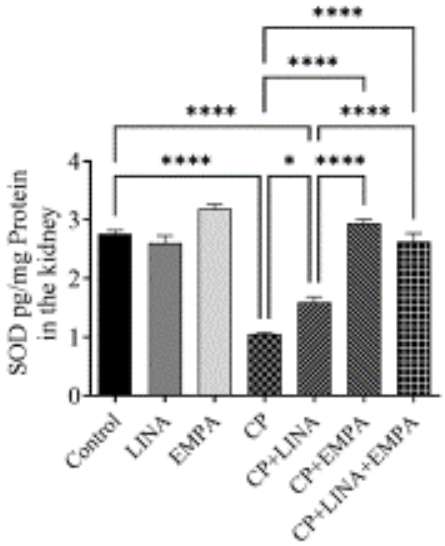


Figure 10 (A) **Figure 10 (B)**

Figure10 : Effect of LINA ,EMPA alone and in combination with CP on SOD

(A) The effect of LINA, EMPA alone and in combination with CP on SOD in the heart. (B) The effect of LINA, EMPA alone and in combination with CP on SOD in the kidney. Note: LINA: Linagliptin; EMPA: Empagliflozin; CP: Cisplatin;SOD :Superoxide dismutase ; N= 7; * $p < 0.05$, ** $p < 0.01$, ***$p < 0.001$ and ****$p<0.0001$ compared to the control /CP group .

Figure 10 (A) revealed a remarkable reduction of heart SOD levels in the CP ($p<0.0001$) and CP+LINA ($p<0.001$)groups compared to the control group. While, significant increase of SOD level was noted in CP+EMPA($p<0.001$) and CP+LINA+EMPA($p<0.0001$) groups compared to the CP group. In kidney tissue, SOD levels significant increase in the CP($p<0.0001$) and CP+LINA ($p<0.0001$) groups compared to the control group. On other hand all treated groups CP+LINA($p<0.05$), CP+EMPA($p<0.0001$), and CP+LINA+EMPA ($p<0.0001$) showed significant increase in SOD level compared to CP group (Figure10B).

3.5 Effect of LINA, EMPA alone, and in combination with CP on oxidative stress parameter (Catalase) in the heart and kidney

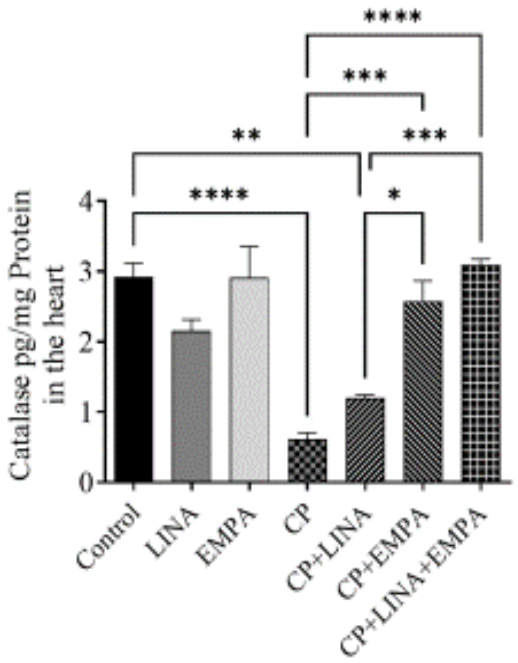


Figure 11 (A)

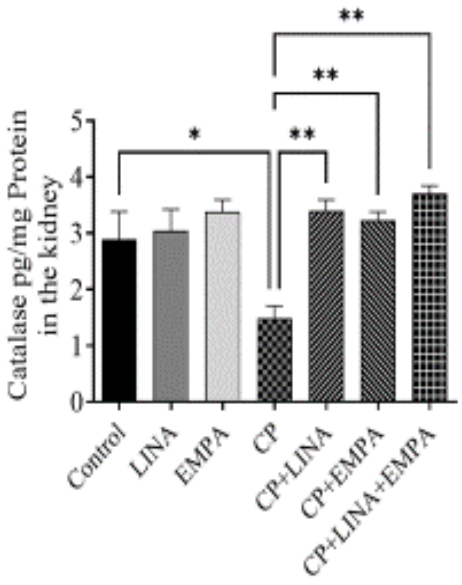


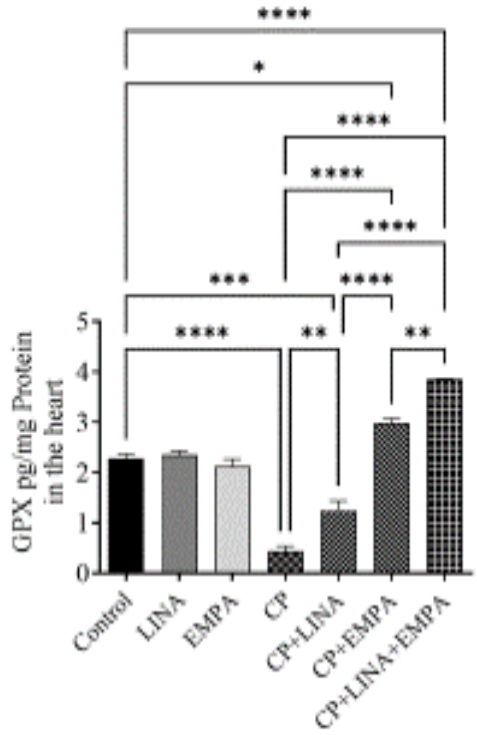
Figure 11(B)

Figure11 : Effect of LINA, EMPA alone and in combination with CP on catalase

(A) The effect of LINA, EMPA alone and in combination with CP on catalase in the heart. (B) The effect of LINA, EMPA alone and in combination with CP on catalase in the kidney. Note: LINA: Linagliptin; EMPA: Empagliflozin; CP: Cisplatin; N= 7;. $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and ****$p<0.0001$ compared to the control /CP group.

(Figure 11A) illustrates that cardiac catalase level was considerably decreased in the CP ($p< 0.0001$) and CP+LINA($p<0.01$) groups in comparison with the control group. In contrast, a considerable rise obtained in the CP+EMPA and CP+LINA+EMPA groups when compared with the CP group ($p<0.0001$, $p<0.001$, respectively). (Figure11B) shows that the administration of CP in the CP group resulted in a marked decrease in Kidney catalase when compared to the control group($p<0.05$). On the contrary, Kidney catalase was significantly increased in treated groups of CP+LINA, CP+EMPA, and CP+LINA +EMPA ($P<0.01$) when compared with the CP group .

3.6 Effect of LINA, EMPA alone, and in combination with CP on oxidative stress parameter (glutathione peroxidase) in the heart and kidney



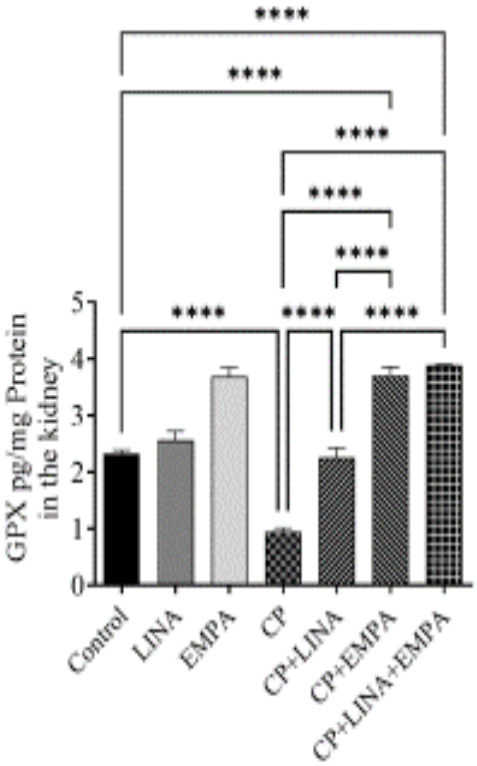


Figure 12(A) **Figure 12 (B)**

Figure 12: Effect of LINA, EMPA alone and in combination with CP on GPX
(A)The effect of LINA, EMPA alone and in combination with CP on GPX in the heart (B) The effect of LINA, EMPA alone and in combination with CP on GPX in the kidney . Note: LINA: Linagliptin; EMPA: Empagliflozin; CP: Cisplatin;GPX:glutathione peroxidase ; N= 7. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and ****$p<0.0001$ compared to the control /CP group.

(Figure 12A) reveals that GPX levels in cardiac tissues decreased significantly in the CP and CP+LINA groups compared to the control ($p<0.0001$, $p<0.001$, respectively). However, the treated groups CP+LINA($p<0.01$), CP+EMPA($p<0.0001$), and CP+LINA +EMPA ($p<0.0001$) exhibited a remarkable increase in cardiac GPX levels compared with the CP group. The GPX activity in kidney tissue shows a significant decrease in the CP group compared to the control group ($P<0.0001$), while this activity was considerably increased in CP+LINA, CP+EMPA, and CP+LINA +EMPA groups compared to the CP group ($p<0.0001$) (Figure 12B).

3.7 Effect of LINA, EMPA alone, and in combination with CP on oxidative stress parameter malondialdehyde in the heart and kidney

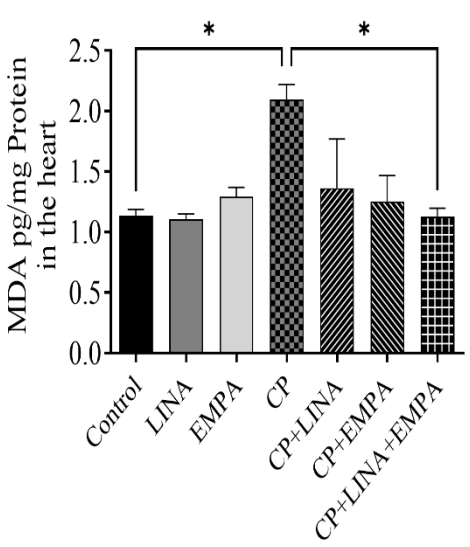


Figure 13 (A)

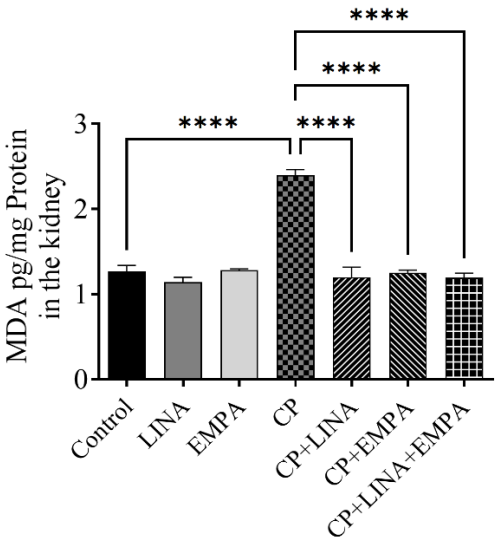
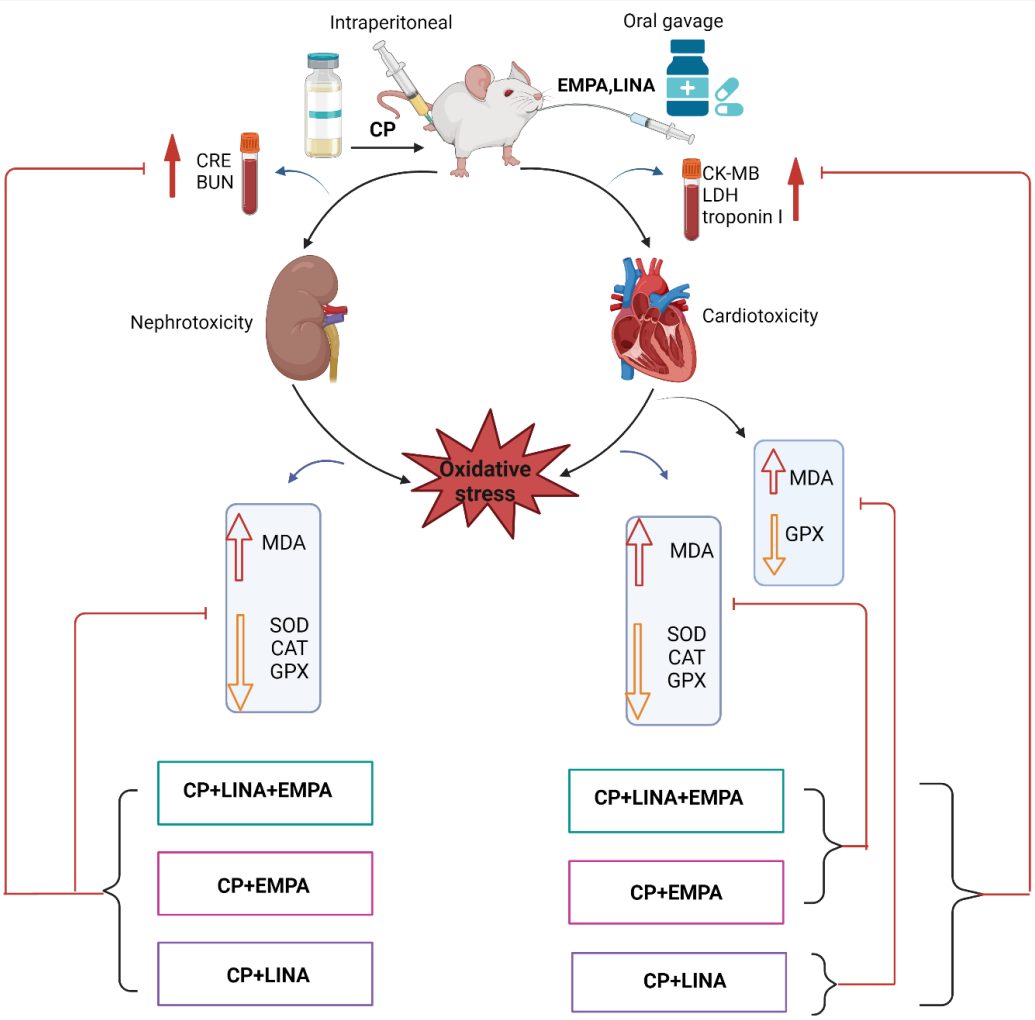


Figure 13 (B)

Figure 13 :Effect of LINA, EMPA alone and in combination with CP on MDA

(A) The effect of LINA, EMPA alone, and in combination with CP on MDA in the heart(B) The effect of LINA, EMPA alone, and in combination with CP on MDA in the kidney. Note: LINA: Linagliptin; EMPA: Empagliflozin; CP: Cisplatin; MDA: malondialdehyde; N= 7; * $p < 0.05$ and ****$p<0.0001$ compared to the control /CP group.

(Figure 13A) shows rise in the level of cardiac MDA in CP group compared to the control group ($p<0.05$). In contrast, the MDA level was noticeably decreased in the (CP+LINA +EMPA) group compared to the CP group ($P<0.05$), in addition to the non-significant reduction in CP+LINA and CP+EMPA groups. Evaluation of MDA level in the kidney was more pronounced. The data revealed a marked increase in the CP group compared to the control group($P<0.0001$), while a notable reduction in MDA level was observed in the CP+LINA, CP+EMPA, and CP+LINA +EMPA groups compared to the CP group ($P<0.0001$) (Figure 13B).



CP: cisplatin ,EMPA : empagliflozin ,LINA : linagliptin ,CRE : creatinine ,BUN : blood urea nitrogen
CK-MB : creatine kinase -myocardial band , LDH : lactate dehydrogenase
MDA : malondialdehyde , SOD : superoxide dismutase ,CAT : catalase ,GPX : glutathione peroxidase

Figure 14 : summary of the results

CHAPTER IV:
Discussion

4. DISCUSSION

Despite significant advancements in cancer chemotherapy regimens that have enhanced the quality of life for long-term survivors, these medications are related with many toxic adverse effects that resulted in noticeable deterioration of renal and cardiac function in these individuals. CP remains an efficacious anticancer drug. Nonetheless, its deleterious side effects, including nephrotoxicity, cardiotoxicity, myelotoxicity, ototoxicity, hepatotoxicity, and gastrointestinal toxicity, restrict its application in the treatment of malignant malignancies [51,52].

The current study aimed to examine the ameliorative effects of EMPA and LINA versus cardiorenal injury induced by CP, assess whether the combination of EMPA and LINA synergistically mitigates cardiorenal injury caused by CP, and explain the molecular mechanisms underlying CP-induced nephrotoxicity and cardiotoxicity.

Nephrotoxicity induced by CP was due to the toxic effect of cisplatin on renal tubules, which impairs particular membrane transport systems, resulting in increased excretion of certain important endogenous chemicals. This leads to a significant drop in excretory systems within the kidney, increasing the buildup of byproducts such as urea, nitrogen, and creatinine [53]. This study revealed that CP induced nephrotoxicity, evidenced by a considerable rise in creatinine and BUN levels related to the control rats. Our findings are in line with that of Gilani SJ and colleagues [54] carried out a model of cisplatin-induced nephrotoxicity and found that the cisplatin-treated rats had substantially elevated levels of several serum chemistry parameters such as BUN, and creatinine levels ($p < 0.05$) . A parallel effect was previously documented by the study of lsuhaibani [55] carried out a model of cisplatin-induced nephrotoxicity and found that the cisplatin-treated rats exhibited a significant increase in serum levels of urea, uric acid, and creatinine as compared to the control group .

Cardiotoxicity generated by CP was evaluated by measuring blood indices indicative of cardiac injury and myocardial damage [56]. CP may damage cell membranes, causing the release of intracellular proteins like cardiac troponin I, LDH, and CK-MB.[8] In the current study, CP treatment significantly raised LDH, CK-MB, and troponin I levels indicating myocardial injury compared to control rats.

Our findings are in line with that of Matovic and colleagues [57] carried out a model of cisplatin-induced cardiotoxicity and found that CP administration resulted in a threefold increase in CK and LDH when compared to control rats . A parallel effect was previously documented by the study of Saleh et al[85] carried out a model of cisplatin-induced cardiotoxicity and found that CP injection caused a prominent elevation in serum troponin T content as well as LDH and CK-MB activities to 218 %, 159 % and 176 % respectively, in comparison with the normal control group.

These biochemical changes were significantly attenuated by LINA and EMPA pretreatment, suggesting that LINA and EMPA pretreatment could effectively counteract CP-induced heart and kidney cell injury. This finding concurs with the report of Mishriki and colleagues [59] which reported EMPA considerably reduce nephrotoxicity caused by 5-Fluorouracil(5-FU). It showed that the Rat groups received 5-FU+EMPA as prophylaxis or treatment, exhibited significant ($P<0.001$) reduction in serum creatinine, urea, uric acid, and significant ($P<0.01$) increase. in serum albumin concentration when compared to 5-FU treated group . Furthermore, George et al. [60] described that EMPA ameliorated Alleviates Carfilzomib-Induced Cardiotoxicity in Mice, resulting in Carfilzomib EMPA-treated mice demonstrating a 1.46-fold significant reduction in troponin I serum levels and reduced serum CK-MB levels by 1.49-fold. As regards LINA, previously documented by the study of Nady and colleagues [61] where Lina has offered protection against tacrolimus-induced renal injury, Lina co-treatment remarkably decreased the levels of Scr and BUN/Scr ratio, to 35%, 28%, respectively. Ishizue et al. [62] carried out a model of isoprenaline induced MI in rats and found that linagliptin decreased the elevated serum cardiac troponin I caused by isoprenaline injection. Additionally, Sravanthi et al. [63] who stated that linagliptin treatment reduced the levels of CKMB and LDH in diabetic rats, providing support for tissue protection.

MDA, a biomarker for lipid peroxidation, serves as a reliable signal for evaluating oxidative stress [64]. The cellular antioxidant enzymatic defense against free radicals greatly enhances the avoidance of oxidative stress damage [65]. This was validated in this study by a notable decrease in antioxidant markers in heart and kidney tissue (SOD, GPX, and CAT) and the rise in cardiac and renal MDA levels in the CP group.CP induces oxidative stress, likely via its interaction with the thiol group of glutathione, resulting in the creation of a highly reactive species that, upon reacting with oxygen molecules, generates ROS. Furthermore, this process depletes levels of antioxidant enzymes such as SOD, GPX, and CAT, ultimately disrupting the cellular oxidation/antioxidant equilibrium and precipitating intracellular oxidative stress [66,67].

In this study, 10 mg/kg of EMPA orally improved renal and cardiac function parameters in the CP+EMPA group. In recent years, it has become more acknowledged that SGLT-2i possesses tissue-protective benefits in addition to their systemic glucose-lowering capabilities. Prior clinical investigations have shown that EMPA maintains kidney protective efficacy even with diminished Glomerular Filtration Rate [68,69]. EMPA received approval for the treatment of heart failure in both diabetic and non-diabetic individuals [70]. Besides regulating hyperglycemia, SGLT2i also protects the kidneys and heart by diminishing oxidative stress, inflammatory processes, sympathetic nerve activity, and autophagy. The pleiotropic effects of SGLT2 inhibitors may account for their therapeutic

effectiveness in treating non-diabetic renal disorders [71] and in mitigating cardiovascular incidents, especially heart failure, in individuals with and without diabetes [72]. EMPA lowered oxidative stress biomarkers in the cardiac tissue of diabetic mice, a result of decreased NOX4 expression due to the activation of nuclear factor erythroid 2-related factor 2/antioxidant response elements (Nrf2/ARE) Signaling cascade [73].

This study demonstrates that EMPA treatment significantly mitigated CP-induced oxidative damage, as indicated by the restoration of renal and cardiac SOD, CAT, and GPX enzyme activity, a noteworthy decrease in renal MDA levels, and a non-significant reduction in cardiac MDA concentration.

Among the worldwide marketed DPP-4 inhibitors, linagliptin is notably significant due to its pleiotropic renoprotective properties, being the sole medication primarily excreted via non-renal routes. Therefore, dose adjustment is unnecessary for chronic renal disease [74,75] .

Treatment of CP rats with LINA improved renal and cardiac biomarkers as an indicator of its Cardiorenal protective effect. The antioxidant capabilities of Lina have not been disclosed in relation to other DPP-4 i. Unlike other drugs in this family, Lina possesses a xanthine backbone and is capable of inhibiting xanthine oxidase, an enzyme implicated in purine metabolism that produces ROS [76] . Lina mitigates ROS by activating the Nrf2/HO-1 signaling pathway in immortalized rat renal tubular epithelial cell lines (NRK52E cells) within a model of AKI caused by lipopolysaccharide endotoxic shock [77] .

The current study's findings showed that administering LINA treatment caused a considerable rise in cardiac GPX and renal SOD, CAT, and GPX and a non-significant increase in cardiac SOD and CAT and a significant reduction in renal MDA compared to the CP group. While cardiac MDA was reduced, it was without statistical significance. Several studies demonstrated the cardioprotective effects of LINA [78, 79]. However, this study is the first to appraise LINA's cardioprotective effect on nondiabetic rats treated with CP. Moreover, the current study uniquely evaluates the ameliorative impact of combined LINA +EMPA on CP-induced cardiorenal injury. The combination reveals a significant rise in cardiac and renal SOD, CAT, and GPX activities and a significant decrease in cardiac and renal MDA levels which suggests the mitigating effect of these drugs in cardiorenal injury caused by CP.

CHAPTER V:
Conclusion

5. CONCLUSION

This study revealed that empagliflozin and linagliptin (solely and in combination) improved cardiorenal function and oxidative stress biomarkers in rats treated with cisplatin, which may reflect their ameliorative effect on cardiorenal toxicity induced by cisplatin. Therefore, they could be promising approaches to mitigate cardiorenal damage associated with cisplatin treatment. Further research is required to substantiate the present study findings as well as to fully elucidate more mechanisms underlying these effects.

CHAPTER VI:
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التأثير التحسيني للإمباجليفلوزين والليناجليبتين على السمية الكلوية والقلبية الناجمة عن السيسبلاتين

إعداد

ملاك صلاح الحربي

الملخص العربي

يعد السيسبلاتين أحد أكثر أدوية العلاج الكيميائي فعالية والتي تستخدم لعلاج أنواع مختلفة من السرطان. ومع ذلك، فإن استخدامه مقيد بسبب آثاره الجانبية مثل السمية العصبية، والسمية الكلوية، وسمية القلب. إن تطور مثبطات ناقل الصوديوم الجلوكوز -2 (SGLT2)، ومثبطات ديببتيدايل ببيتيديز -4 (DPP-4i) في علاج داء السكري من النوع الثاني يوفر خيارًا جديدًا لتحسين السمية المرتبطة بعلاج السيسبلاتين. تم توثيق التأثيرات الوقائية للإمباجليفلوزين (SGLT2i) والليناجليبتين (DPP-4i) على وظائف القلب والأوعية الدموية والكلى جيدًا. أن إمباجليفلوزين وليناجليبتين قد يخففان من سمية القلب والكلى المرتبطة بالعلاج بالسيسبلاتين.

لذلك ستقوم هذه الدراسة بتقييم التأثيرات الوقائية للإمباجليفلوزين والليناجليبتين ضد إصابة القلب والكلى الناجمة عن السيسبلاتين، ولتقييم ما إذا كان مزيج إمباغليفلوزين والليناجليبتين يعملان بشكل تآزري لتعديل وظائف الكلى والقلب والأوعية الدموية بعد تناول السيسبلاتين، ولتحديد الآلية الجزيئية للسيسبلاتين على الجسم. السمية الكلوية وسمية القلب .

سيتم استخدام تسع واربعون من فئران ويستار الذكور في هذه الدراسة وتقسيمهم عشوائياً إلى سبع مجموعات متساوية على النحو التالي: مجموعة المتحكمة، مجموعة السيسبلاتين، 10ملجم/كجم إمباغليفلوزين و إمباغليفلوزين مع سيسبلاتين،3 ملجم/كجم ليناجليبتين ، ليناجليبتين مع سيسبلاتين، إمباجليفلوزين وليناجليبتين عن طريق الفم بالإضافة إلى سيسبلاتين لمدة 10 يوم ، وفي اليوم الاول يتم حقن سيسبلاتين 20 ملغم/كغم في منطقة الغشاء البريتوني مرة واحدة .

سيتم تقييم المؤشرات الحيوية الكلوية، والمؤشرات الحيوية للقلب، ومعلمات الإجهاد التأكسدي. سيتم تحليل عينات القلب والكلى بحثًا عن الإجهاد التأكسدي.

المملكة العربية السعودية

وزارة التعليم العالي

جامعة القصيم

كلية الصيدلة

قسم علم الأدوية والسموم

التأثير التحسيني للإمباجليفلوزين والليناجليبتين على السمية الكلوية والقلبية الناجمة عن السيسبلاتين

رسالة مقدمة الاستكمال متطلبات الحصول على درجة الماجستير في علم الأدوية

إعداد

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قر بأنني قد التزمت بضوابط جامعة القصيم، وأنظمتها، واللوائح المتعلقة بإعداد الرسائل العلمية، وقد قمت شخصيا بإعداد رسالتي؛ وذلك بما ينسجم مع الأمانة العلمية، والمعايير الأخلاقية المتعارف عليها دوليا في كتابة الرسائل العلمية والبحث العلمي. كما أقر بأن رسالتي هذه غير منقولة، أو مسائلة، أو منتحلة من رسائل، أو كتب، أو أبحاث، أو أي منشورات علمية تم نشرها، أو تخزينها في أي وسيلة إعلامية، ولم يسبق تقديمها للحصول على أي درجة علمية أخرى، وان هذه النسخة المودعة هي النسخة النهائية بعد اجراء التعديلات المطلوبة من قبل أعضاء لجنة المناقشة وعليه أتحمل كامل المسؤولية على ذالك.

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