ORIGINAL ARTICLE

CONTENTS

Carbenoxolone attenuates renal ischemia-reperfusion injury in rats by modulating inflammatory cytokines, oxidative stress markers, and apoptotic pathways

Munther Abosaooda[1], Qayssar Joudah Fadheel[2]
[1]COLLAGE OF PHARMACY, THE ISLAMIC UNIVERSITY, NAJAF, IRAQ
[2]DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY, COLLEGE OF PHARMACY, UNIVERSITY OF BABYLON, BABYLON, IRAQ

ABSTRACT

Aim: This study investigates the protective effects of Carbenoxolone (Cbx) in attenuating renal injury through the modulation of the FOXO3 gene.

Materials and methods: Twenty-eight adult male Wistar Albino rats were randomly divided into four groups (N=7): Sham (laparotomy without ischemia), Control (bilateral renal ischemia for 30 min followed by 2 h reperfusion), Vehicle (DMSO injection prior to IRI), and Carbenoxolone-treated (Cbx injection three days before IRI).

Results: Tissue levels of TNF-α, IL-1β, MDA, Caspase-3, and KIM-1 were significantly elevated in the control and vehicle groups, while GSH levels were reduced. Cbx treatment significantly decreased TNF-α, IL-1β, MDA, Caspase-3, and KIM-1 while increasing GSH levels, indicating enhanced antioxidant defense. FOXO3 expression was notably lower in the Cbx group compared to the control and vehicle groups. Histopathological analysis further confirmed reduced renal damage in Cbx-treated rats. These findings suggest that Cbx exerts renoprotective effects by mitigating oxidative stress, inflammation, and apoptosis.

Conclusions: Carbenoxolone significantly reduces RIRI-induced kidney damage, demonstrating its potential as a therapeutic agent for renal ischemic injuries.

KEY WORDS: Carbenoxolone, RIRI, IL-1β, TNFα, KIM-1, FOXO3

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ABBREVIATIONS

Cbx - Carbenoxolone,
I/R - ischemia reperfusion
KIM-1 - kidney injury molecule 1
TNFα - tumor necrosis factor alpha,
IL-1β - interleukin -1 beta,
FOXO3 - Forkhead Box O3,
DMSO - dimethylsulfoxide

INTRODUCTION

Renal ischemia-reperfusion injury (RIRI) is a major cause of acute kidney injury (AKI), with the inflammatory response playing a critical role in its pathogenesis [1-2]. Following re-oxygenation, ischemia-reperfusion injury occurs primarily due to the excessive production of reactive oxygen species (ROS), leading to various cellular outcomes, including programmed cell death, necrosis, and the release of injury-associated factors that serve as initiators of the inflammatory response [3-4]. Apoptosis has two diverse mechanisms: the first pathway (extrinsic) is activated by the death receptors, a subgroup of the tumor necrosis factor (TNF) receptor family and the intrinsic pathway which focuses on mitochondria [5]. The renal injury resulting from ischemia-reperfusion is a dynamic process involving complex interactions among inflammatory mediators, oxidative stress, and lipid peroxidation, all of which exacerbate tissue damage [6]. The PI3K/Akt signaling pathway plays a significant role in this process by phosphorylating FOXO3, a transcription factor involved in regulating apoptosis, oxidative stress response, and cell cycle progression [7]. Oxidative stress, characterized by the excessive generation of ROS, contributes to renal cell damage by triggering lipid peroxidation and depleting crucial antioxidants such as glutathione (GSH). The accumulation of malondialdehyde (MDA), a lipid peroxidation by-product, serves as a marker of oxidative stress and correlates with the severity of renal injury. These reactive oxygen species may lead to damage of mitochondrial functional, energy imbalances and cause apoptosis [8]. Additionally, inflammatory cytokines, including tumor

necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), are key mediators in the pathogenesis of AKI, further amplifying the inflammatory response. Renal function is commonly assessed through biomarkers such as kidney injury molecule (KIM-1), which provide insight into the extent of ischemia-reperfusion injury[4] Multifunctional compounds with antioxidant, anti-inflammatory, and anti-inflammatory capabilities serve as the best defenses in cases of kidney damage. The protective properties of antioxidants can be explained by their ability to restore intracellular processes related to oxidative damage kinetics [9-10]. In addition, antioxidant therapy can protect against the oxidative damage caused by infrared radiation. Antioxidant compounds have been found to restore intracellular processes related to oxidative damage, potentially contributing to their protective properties [11]. Inflammation caused by ischemia-reperfusion injury (IRI) can lead to further renal damage, but protecting against this occurrence is possible [12]. Pro-inflammatory cytokines, such as tumor necrosis factor (TNF-α), interleukin-1-B (IL-1B), and interleukin-6 (IL-6), play a primary role in renal disease [13-14] This study aimed to investigate the nephron-protective effects of Carbenoxolone on renal ischemia-reperfusion injury in rats by measuring the following parameters: Kidney injury molecule 1 (KIM-1), interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), GSH, MDA, Caspase-3, Foxo3 gene expression and histological examination.

MATERIALS AND METHODS

PREPARATION OF ANIMALS

In this study, 28 adults male Wistar Albino rats aged more than 20 weeks and an average weight of 300±50 g were used. All animals had free access to food and water and were subjected to a 12:12 light-dark cycle. The temperature and humidity were controlled at 25 °C and 60-65%, respectively. The rat handling, experiments, and tests complied with the Ethical Conduct for Use of Animals guidelines and regulations. The animals were housed at the animal house of the College of Sciences, University of Kufa. The materials were procured from Medchem express, Sunlong Technology Lab, China and Macrogen/ Korea.

ETHICAL STATEMENT AND APPROVAL

The study was done in the department of pharmacology and toxicology, Faculty of Pharmacy, University of Kufa and in Middle Euphrates Unit for Cancer Researches, Faculty of Medicine, University of Kufa. The study was accepted by Committee center of Bioethics in the University of Kufa and its representative in Faculty of Pharmacy. Whole procedures were done according to the recommendations of the Committee.

All procedures involving the handling and experimentation on rats, as well as the conducted tests, were carried out in compliance with the applicable guidelines and regulations for the ethical use of animals, Kufa University (20545 in 29/8/2024). The animals were housed in the animal facility at the College of Sciences, University of Kufa.

CARBENOXOLONE

The pure powder of Carbenoxolone disodium was gutted from medchemexpress, USA Company. Formal name: (3β,20β)-3-(3-carboxy-1-oxopropoxy)-11-oxo-olean-12-en-29-oic acid, disodium salt Chemical name: Carbenoxolone disodium, Chemical Formula: $C_{34}H_{48}O_7 \cdot 2Na$, CAS Number: 7421-40-1, Molecular Weight: 614.7, Physical Description: A crystalline solid, Color: White to off-white, Solubility: In DMSO: 12.5 mg/mL. DMSO was considered the standard vehicle for preparation this drug before use. The dose of drug that used was 200mg\kg of rat weight intraperitoneal [15].

EXPERIMENTAL DESIGN

In this study, Wistar Albino rats were randomly selected and divided into four groups, each consisting of seven rats, subjected to different handling procedures. The sham group served as the negative control and underwent no IRI procedure. The control group underwent bilateral renal ischemia by clamping the renal pedicles for 30 minutes, serving as the positive control. The vehicle-treated group received an intraperitoneal injection of DMSO given every 24 hr. for 2 consecutive days (before the day of decapitation) and 1 h before ischemia induction on third day and undergone bilateral renal ischemia for 30 min and reperfusion for 2 hours. Rats in the last group received Cbx. intraperitoneal injection 200 mg/kg given every 24 hr. for 2 consecutive days (before the day of decapitation) and 1 h before ischemia induction on third day.

RENAL ISCHEMIA REPERFUSION INJURY RAT MODEL

Rats were anesthetized, maintained at 37°C, and underwent midline incisions to expose renal pedicles. Ischemia was induced by clamping both pedicles for 30 minutes, followed by reperfusion. Hydration was ensured with sterile saline. After suturing, bilateral nephrectomy was performed, and kidneys were washed

Table 1. Primers used in this study

Host	Gene		5′-3′	Product (bp)	Accession number	Reference
Rattus	FOXO-3	F	AAAGGGGAAATGGGCAAAGC	83	XM_032888496.1	Nayakanti, 2022
		R	GGCTGAGAGCAGATTTGGCA			
Rattus	GAPDH	F	ATGACTCTACCCACGGCAAG	89	NM_017008	Kunst et al., 2012
		R	CTGGAAGATGGTGATGGGTT			

with precooled PBS to remove blood. Finally sacrificed the rat by heart puncture [16]. The left kidney was cut sagittal into 2 halves. The first half was kept in deep freeze for bimolecular assessment. While the second half was inserted in 10% formalin then embedded in paraffin for histopathological.

PREPARATION OF TISSUE FOR MEASUREMENT OF IL1-B, TNF-A, GSH, MDA, CASPASE-3 AND KIM-1 BY ELISA

At the end of the surgical procedure, the left kidney was taken from the dead rat and rinsed by ice-cold normal saline to remove any blood clot and then dissected to two parts. One section was taken and homogenized with a high intensity ultrasonic liquid processor in 1:10 (w/v) phosphate buffered saline that contained 1% Triton X-100 and a protease inhibitor cocktail. The homogenate was centrifuged at 26,310 x g for 20 min at 4°C. The supernatant was collected for determination of IL1-β, TNF-α, GSH, MDA, KIM-1 and Caspase-3 by ELISA technique (Bio-Elisa Reader-BioTek Instruments, USA).

TISSUE SAMPLING FOR HISTOPATHOLOGY ANALYSIS AND DAMAGE SCORES

The left kidney tissue sample was fixed in 10% formalin, processed through alcohol dehydration, xylene clearing, and paraffin embedding. Thin sections (5 µm) were stained with Hematoxylin and Eosin (H&E) for histopathological analysis. A blinded investigator assessed tissue damage using a quantitative scoring system under light microscopy at 40× magnification. Renal tubular injury was evaluated based on epithelial swelling, vacuolar degeneration, brush border loss, necrosis, and cast formation. The histological damage was categorized as follows: Score 0 (normal), Score 1 (<25% damage), Score 2 (25%–50%), Score 3 (50%–75%), and Score 4 (>75%).

ASSESSMENT OF TISSUE FOXO3 GENE EXPRESSION BY RT-QPCR

1. Total RNA Extraction Using Easy-spin™ (DNA free) Total RNA Extraction Kit
2. cDNA Synthesis (Using Add Script cDNA Synthesis Kit)
3. Preparation of Primers
4. Primers Used in this Study (Table 1)[17-18]
5. Protocol of GoTaq® RT-qPCR System for Real-Time qPCR (Gene expression assay).

STATISTICAL ANALYSIS

Statistical analyses were performed using GraphPad Prism and SPSS 28.0 for window. Inc. Data were expressed as mean ± SD. Analysis of variance (ANOVA) was used for the multiple comparisons among all groups followed by post–hoc tests using Bonferroni method. For the histopathological renal changes, the Kruskal-Walli's test was used to assess the statistical significance of difference across multiple groups in total severity score (mean score) for histopathological renal changes. In all tests, P≤0.001 was considered statistically significant.

RESULTS

EFFECTS OF CARBENOXOLONE ON KIDNEY INJURY MOLECULE-1 (KIM-1)

The renal tissue level of (KIM-1) in control and vehicle groups were significantly ($p<0.001$) higher than that in the sham group, alternatively, the level of KIM-1 in the Cbx treated group is significantly ($p<0.001$) lower than that in the control group (Fig 1A).

EFFECT OF CARBENOXOLONE ON THE OXIDATIVE STRESS MARKER GLUTATHIONE (GSH)

The mean GSH levels were significantly lower in the Control and control vehicle group compared to the Cbx treated groups ($P<0.001$) while slightly decreased when compare with the sham group (Fig 1B)

EFFECT OF CARBENOXOLONE ON THE OXIDATIVE STRESS MARKER GLUTATHIONE (GSH)

The renal tissue level of MDA in control and vehicle groups were significantly $p<0.001$ higher than that in

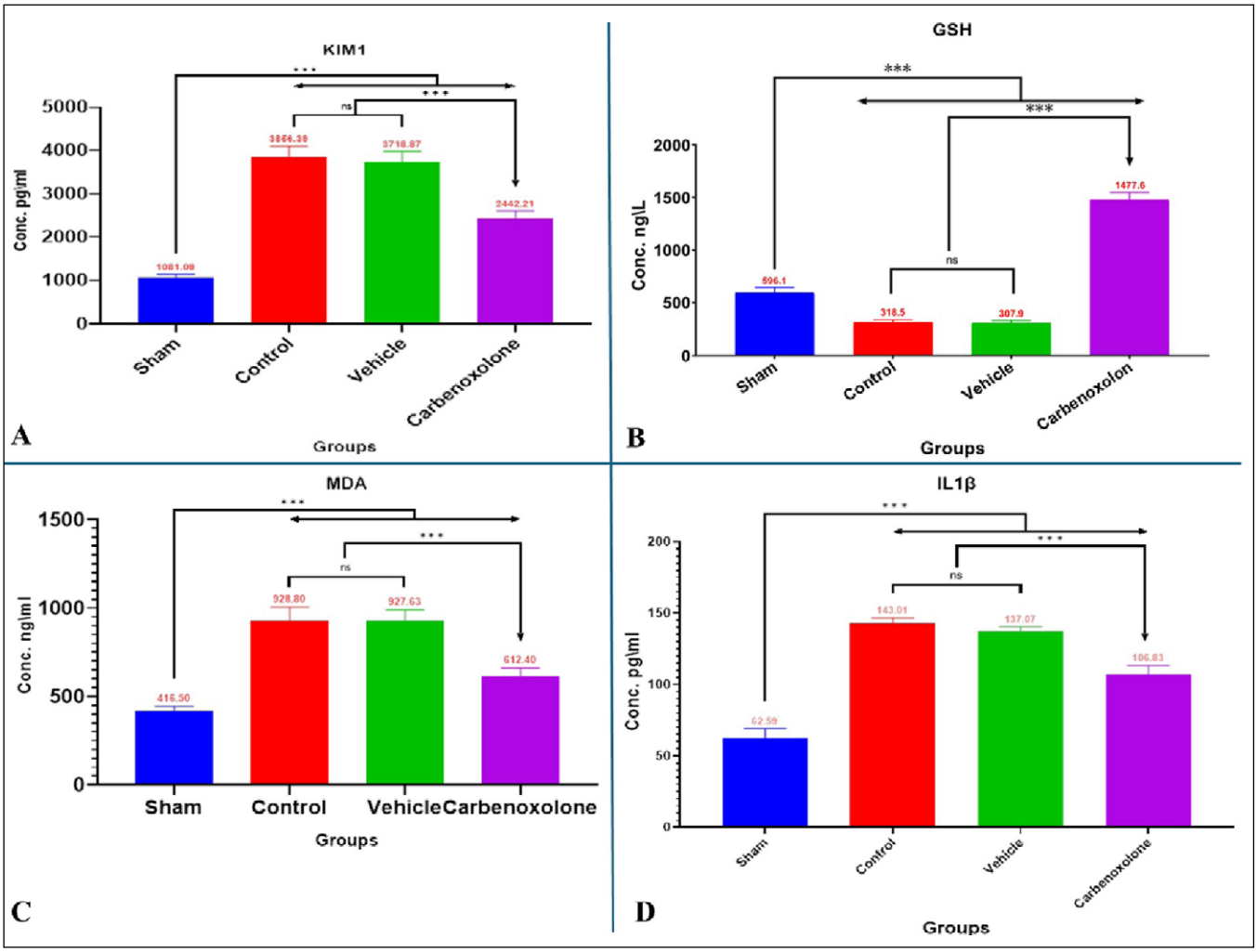

Fig 1. A. The statistical analysis of KIM1 concentrations mean (pg/ml) in renal tissues in the four experimental study groups at the end of the study (No of rats = 7 in each study group) Sham group *vs.* vehicle & control groups, ***P. value < 0.001. Cbx. *vs*. vehicle & control groups, ***P. value < 0.001, **B.** The statistical analysis of GSH concentrations mean (ng/L) in renal tissues in the four experimental study groups at the end of the study (No of rats = 7 in each study group). Sham group *vs.* vehicle & control groups, ***P. value < 0.001. Cbx. *vs.* vehicle & control groups, ***P. value < 0.001**,* **C.** The statistical analysis of MDA concentrations mean (ng/ml) in renal tissues in the four experimental study groups at the end of the study (No of rats = 7 in each study group). Sham group *vs*. vehicle & control groups, ***P. value < 0.001. Cbx *vs*. vehicle & control groups, ***P. value < 0.001, **D.** The statistical analysis of IL-1β concentrations mean (pg/ml) in renal tissues in the four animal study groups at the finishing of the research (No of rats = 7 in each study group).
Sham group *vs.* vehicle & control groups, ***P. value < 0.001. Cbx. *vs*. vehicle & control groups, ***P. value < 0.001
Source: Own materials.

the sham group, in contrast, their levels in the Cbx were significantly p<0.001 lower than that in the control group (Fig. 1C).

EFFECT OF CARBENOXOLONE ON THE INTERLEUKIN -1 BETA

There was a significant P<0.001 difference in IL-1β levels among the studied groups. The sham group had the lowest levels than all other groups (P<0.001 for all comparisons). Conversely, IL-1β levels were significantly (P<0.001) lower in the Cbx treated group compared with control groups (Fig. 1D).

EFFECT OF RIRI, CARBENOXOLONE ON THE TUMOR NECROSIS FACTOR-ALPHA

The renal tissue level of (TNF-α) in control and vehicle groups were significantly p<0.001 higher than that in the sham group, On the other hand, their levels in the Cbx were significantly p<0.001 lower than that in the control group (Fig. 2).

EFFECTS OF CARBENOXOLONE ON THE MARKER OF APOPTOSIS, CASPASE3

The renal tissue level of Caspase3 in control and vehicle groups were significantly (p<0.001) higher than that in the sham

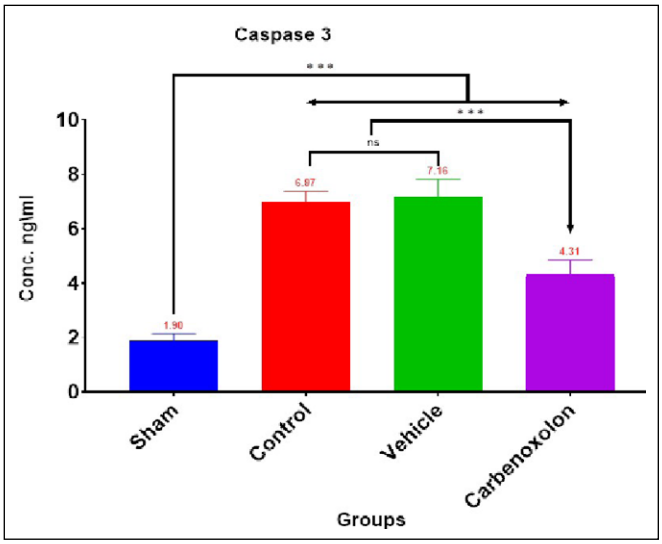

Fig. 2. The statistical analysis of TNFα concentrations mean (ng/L) in renal tissues in the four animal study groups at the end of the study (No of rats = 7 in each study group)
Sham group *vs*. vehicle & control groups, ***P. value < 0.001, Cbx *vs*. vehicle & control groups, ***P. value < 0.001
Source: Own materials.

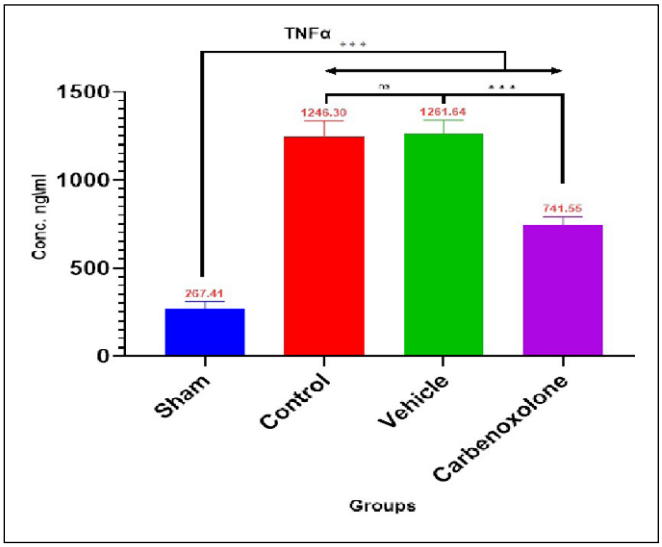

Fig. 3. The statistical analysis of caspase-3 concentrations mean (ng/L) in renal tissues in the four animal study groups at the end of the study (No of rats = 7 in each study group)
Sham group *vs*. vehicle & control groups, ***P. value < 0.001, Cbx vs. vehicle & control groups, ***P. value < 0.001
Source: Own materials.

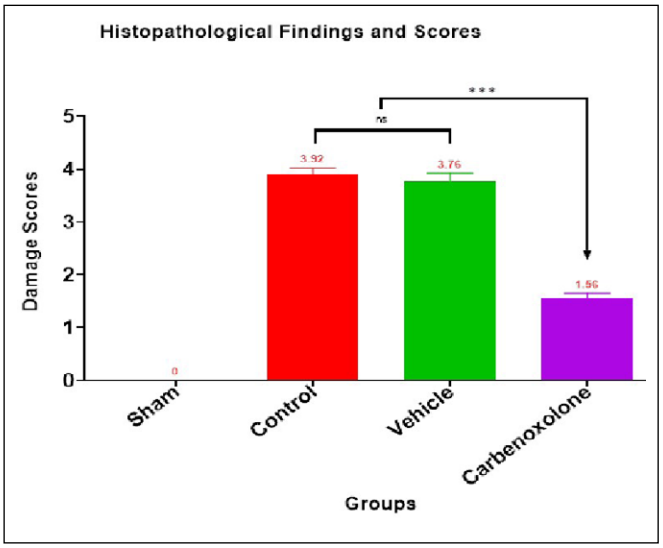

Fig. 4. Mean of fold change of FOXO3 gene expression in renal tissue of the four experimental groups at the end of the study (No of animals = 7 in each group)
Sham group *vs*. vehicle & control groups, ***P. value < 0.001, Cbx *vs*. vehicle & control groups, ***P. value < 0.001
Source: Own materials.

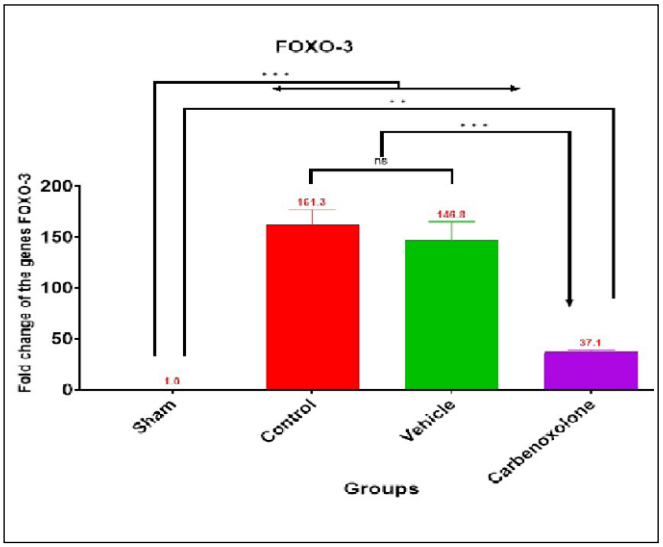

Fig. 5: Score severity mean of renal tissue histopathology of the four experimental groups at the end of the study (No of animals = 7 in each group)
Sham group vs. vehicle & control groups, ***P. value < 0.001, Cbx vs. vehicle & control groups, ***P. value < 0.001
Source: Own materials.

group, On the other hand, their levels in the Cbx were significantly (p<0.001) lower than that in the control group (Fig. 3).

THE EFFECT OF CARBENOXOLONE ON THE INTRACELLULAR SIGNALING (FOXO3) AFTER RIRI BY PCR TECHNIQUE

The renal tissue levels of FOXO3 expression in control and vehicle groups were significantly P<0.001 higher than that in the sham group, Meanwhile, the expression level of FOXO3 in the Cbx treated group is significantly P<0.001 lower than that in the control group (Fig. 4).

HISTOPATHOLOGY FINDINGS

Results of the histopathological examination of renal tissuesc from the four study groups are shown in figures 5, 6A-D, 7.

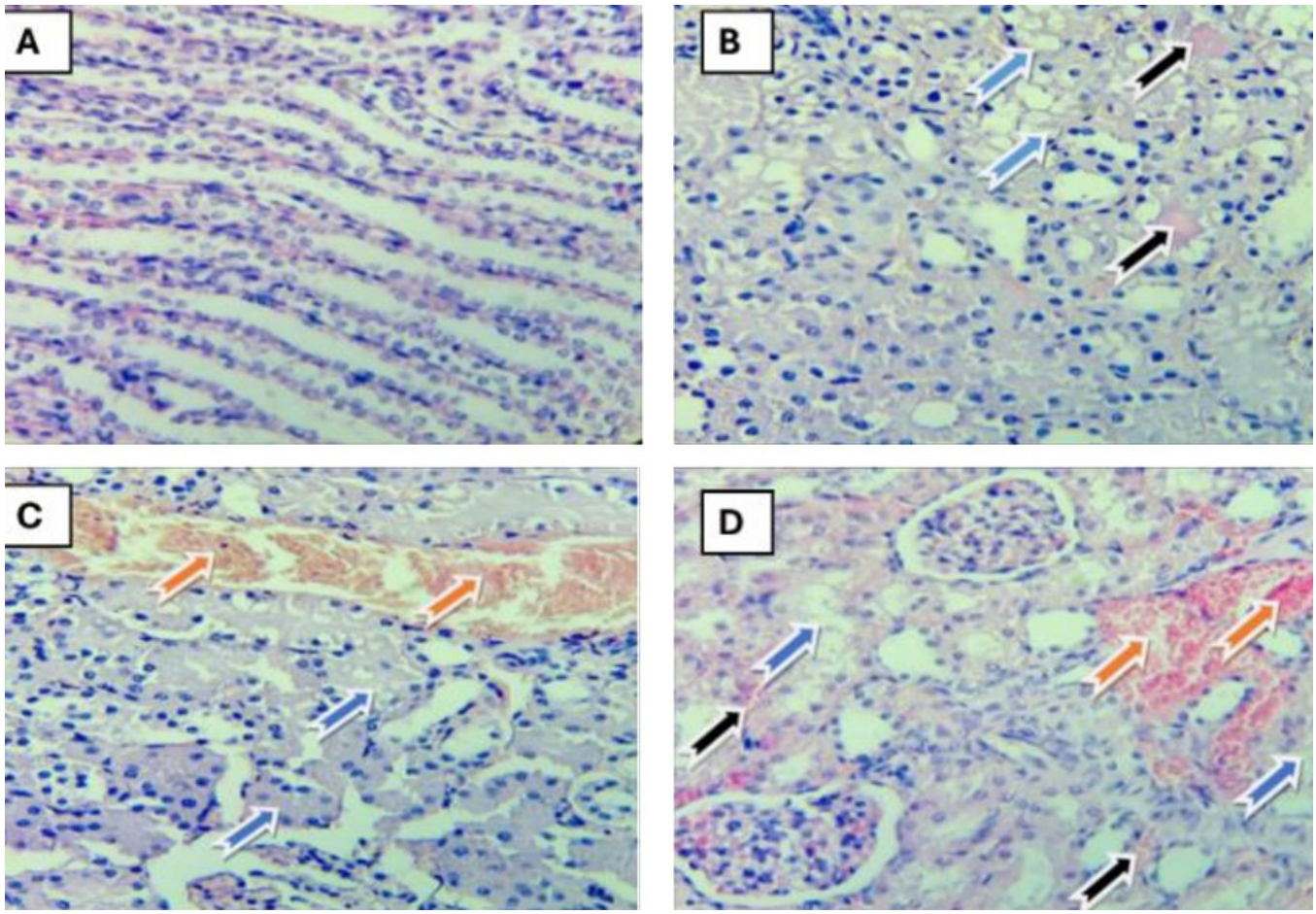

Fig 6. Results of the histopathological examination of renal tissues from the four study groups: **A**-Sham group, a microscopic cross section of left kidney represented normal tissues histology, normal renal tubules, and normal cell size and there is no cast formation, cells edema or loss of brush boarder. Sham group. H & E stain × 400.The mean of the severity score was zero (score severity mean = 0 and represent 0% of damage renal tubules), **B** Control group. Rat kidney, renal tubules with score 4 damage involving 100% of the examined tubules. Cytoplasmic vacuoles (blue arrows) and eosinophilic cast (black arrows). H&E. X400, **C** Control group. Rat kidney, renal tubules with score 4 damage involving 100% of the examined tubules. Vascular congestion (orange arrows) and cytoplasmic eosinophilia (blue arrows). H&E. X400, **D** Vehicle group. Rat kidney, renal tubules with score 4 damage involving 95% of the examined tubules. Cytoplamic swelling and increased cytoplasmic eosinophilia (black arrows), cytoplasmic vacuoles (blue arrows), hemorrhage (orange arrows), H&E. X400
Source: Own materials.

DISCUSSION

Acute kidney injury caused by renal IRI is often associated with high morbidity and mortality and ROS play a critical role in this pathological process [19] ROS can trigger inflammation, increase vasoconstrictors production, and induce tubular cell apoptosis and necrosis [20]. This study investigated the nephroprotective effects of Cbx in RIRI. Cbx treatment reduced Kidney Injury Molecule-1 (KIM-1), indicating renal protection, and attenuated inflammation by lowering TNF-α and IL-1β levels. It also decreased oxidative stress (MDA) while enhancing antioxidant defenses (GSH). Additionally, Cbx downregulated Caspase-3, suggesting anti-apoptotic effects, and modulated FOXO3, implicating its role in cellular survival. These findings highlight Cbx's potential as a therapeutic candidate for AKI management through antioxidant, anti-inflammatory, and anti-apoptotic mechanisms.

EFFECTS OF RIRI, CARBENOXOLONE ON KIDNEY INJURY MOLECULE-1 (KIM-1)

In this experimental work, notably, the groups treated with Cbx exhibited significantly P≤0.001 lower levels of KIM-1 compared to the control and vehicle groups. In contrast, the control and vehicle groups exhibited a marked increase in KIM-1 levels of renal tissue relative to the sham group. These findings are consistent with the results of [21] who demonstrated that KIM-1 is primarily expressed in kidney and drastically upregulated in injured renal tubules. Therefore, KIM-1 is regarded as a sensitive and specific biomarker for kidney inju-

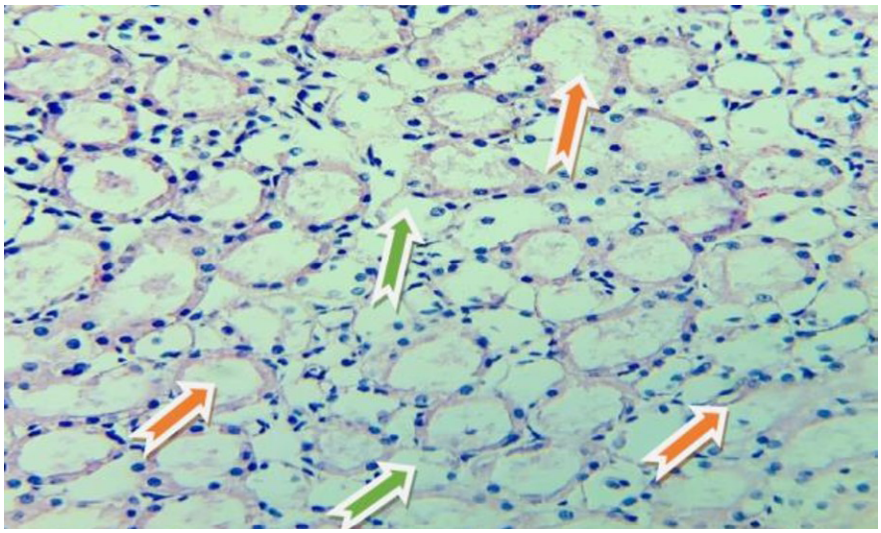

Fig. 7. Cbx treatment group
Rat kidney with score 2 damage involving 35% of the examined tubules. Damaged tubules (orange arrows) and normal tubules (green arrows). H&E. X400
Source: Own materials.

ry which has been approved by the FDA to evaluate nephrotoxicity. Previous studies have established that KIM-1 expression on the apical surface of renal proximal tubule epithelial cells is induced by ischemia and toxic damage [22-23]. The lower KIM-1 levels observed in the treatment groups in this study suggest a potential nephroprotective effect of Cbx in RIRI. To the best of our knowledge, no previous studies have investigated the impact of Cbx on KIM-1 levels in the subject of RIRI. The reduction in KIM-1 levels observed in the present study is likely attributable to the anti-inflammatory and antioxidant properties of this agent. These findings align with the results of [24] who demonstrated that, the antioxidant effect of pioglitazone by reducing the inflammation, oxidative stress, and renal tubular damage through targeting various cellular pathways, including TNF-α, NF-κB signaling, KIM-1, and NGAL pathways.

EFFECTS OF RIRI, CARBENOXOLONE ON TUMOR NECROSIS FACTOR-ALPHA (TNF-A)

The present study revealed that pretreatment with Cbx prior to ischemia induction significantly $P \leq 0.001$ reduces the level of TNF-α in renal ischemic tissues, as compared to the levels of these inflammatory cytokines in the IR control and vehicle groups these findings are consistent with previous studies shown that the levels of TNFα was increased after 30 min of ischemia followed by 2hrs of reperfusion in rats, and there was significant changes in endothelial function and the elevation in this parameter contributes to the endothelial dysfunction [25], also another study in a rat model showed that the level of the TNFα gene was highly elevated in injured kidney tissues in rats that are undergone nephrectomy of the right kidney and ischemia in the left kidney for 45 min then reperfusion [26]. Additionally, [27] demonstrated that the treatment with Cbx could significantly decrease levels of inflammatory factors such as IL-6 (Interleukin 6) and TNF- a (Tumor necrosis factor-a).

EFFECTS OF RIRI, CARBENOXOLONE ON INTERLEUKIN 1 BETA (IL-1B)

In the current study, IL-1β levels were significantly ($P \leq 0.001$) higher in the control group receiving RIRI without treatment, whereas the vehicle group did not differ significantly from the control, suggesting that there were no anti-inflammatory effects. These findings are consistent with earlier research [28], which found that rats that experienced 30 minutes of ischemia and two hours of reperfusion had higher levels of serum creatinine, urea, IL-1β, and caspase-3. In contrast, Cbx-treated groups showed significantly ($P \leq 0.001$) lower levels of IL-1β, indicating that it may have anti-inflammatory effects by inhibiting IL-1β. According to [29], Cbx has anti-inflammatory, antiviral, antitumor, antibacterial, antifibrotic, and neuroprotective qualities. This finding is in line with their findings. By raising IL-10 and decreasing TNF-α, IL-1β, IL-6, and monocyte infiltration, Cbx treatment decreased lung inflammation in pulmonary hypertension models. Cbx reduces IL-1β. By lowering IL-1β, Cbx may mitigate RIRI-induced inflammation and renal damage.

EFFECTS OF RIRI, CARBENOXOLONE ON MALONDIALDEHYDE (MDA)

In this experimental rat model study, the results indicated that the control and vehicle group, which experienced RIRI without any therapeutic intervention, exhibited significantly $P \leq 0.001$ higher levels of MDA as compare with sham group. The increase reflects the oxidative stress induced by ischemic injury. This result are

agreement with those reported by [30] which revealed that RIRI is characterized by a burst of ROS formation, reduction of antioxidants, significantly reduced SOD activity and increased MDA content, resulting in increased oxidative stress and tissue damage. In contrast, the group treated with Carbenoxolone demonstrated significantly P≤0.001 lower level of MDA compared to both the control and vehicle groups. This reduction in MDA levels indicates that Cbx may exert antioxidant effects, potentially through mechanisms that involve the scavenging of free radicals or the enhancement of endogenous antioxidant defenses. These findings align with the work of [31] who demonstrated that the administration of Cbx leads to a significant reduction in MDA levels, a well-established biomarker for lipid peroxidation and oxidative damage. Also, another study reported by [32], demonstrated that treatment with rotenone, a mitochondrial complex I inhibitor, significantly increased ROS levels, this rise in ROS was associated with increased lipid peroxidation. Importantly, co-administration of Cbx effectively mitigated these effects. Cbx treatment not only reduced ROS levels but also reversed the increase in lipid peroxidation in rotenone-treated animals.

EFFECTS OF RIRI, CARBENOXOLONE ON ANTIOXIDANT MARKER GLUTATHIONE (GSH)

GSH is a critical endogenous antioxidant that plays a vital role in protecting cells from oxidative stress by neutralizing free radicals and reactive oxygen species. The [33] found that the level of kidney glutathione in untreated RIRI rats was significantly lower than that of control rats. Other studies confirmed that the level of GSH significantly decreased, which was accompanied with a significant increase in MDA level of kidney tissue following I/R [34]. These findings are consistent with the results in the current study that exhibited significantly lower levels of GSH in the control and vehicle group which underwent RIRI without any therapeutic intervention. Conversely, pretreatment with Cbx displayed significantly P≤0.001 higher levels of GSH compared to both the control and vehicle groups. This increase in GSH levels indicates that Cbx may enhance the antioxidant capacity of renal tissues, potentially through mechanisms that involve the upregulation of GSH synthesis or the reduction of oxidative stress. By increasing GSH levels, Carbenoxolone may help to restore the antioxidant defense system and protect renal tissue from damage associated with RIRI. This is consistent with the conclusions reached by Bhardwaj et al., 2014, [35] that a significant increase P<0.05 in the levels of GSH in case of Cbx alone treated group was observed when compared to the MG-132 treated group in an animal model of Parkinson's disease induced by proteasome inhibitor.

EFFECT OF RIRI, CARBENOXOLONE ON APOPTOTIC MARKER (CASPASE3)

This experimental study confirmed a significant increase in Caspase3 levels in renal tissue of both I/R control and vehicle groups P≤0.001 relative to the sham group following ischemia-reperfusion injury (IRI). While there is no significantly different between control and vehicle groups. These findings supported earlier research by [36] who found that the level of Caspase3 was increased after 30 minutes of ischemia followed by 2 hours of reperfusion in rats. Another studies in a rat model appeared that the level of Caspase3 was highly elevated in injured kidney tissues in rats that undergone ischemia for 45 minutes then reperfusion for 24 hr. and ischemia for 60 minutes then reperfusion for 2 hours, respectively [37-38]. In contrast, this study revealed that the pretreatment with Cbx before ischemia induction can significantly P<0.001 downregulated the expression of caspase-3 in injured renal tissues in comparison with those in both control and vehicle groups. The outcomes in the current study are in agreement with those reported by [39] who demonstrated that pretreatment with Cbx (5-20 µg/ml) significantly reduced reactive oxygen species (ROS) levels. Furthermore, Cbx effectively decreased the expression of cleaved caspase-3 protein in PC12 cells subjected to SGD-induced stress, indicating its potential role in mitigating apoptotic pathways under oxidative conditions. This reduction in Caspase-3 levels indicates that Cbx may exert anti-apoptotic effects, potentially through mechanisms that involve the inhibition of pro-apoptotic signaling pathways or the enhancement of cell survival pathways. By decreasing the levels of Caspase-3, Cbx may help to mitigate apoptosis and protect renal tissue from damage associated with RIRI.

EFFECT OF RIRI, CARBENOXOLONE ON THE MOLECULAR PROTEINS FOXO3

In the present study we found that the renal tissues from the control and vehicle groups, which underwent IRI without any treatment, demonstrated upregulation of FOXO3 gene while Cbx treated group showed downregulation of Foxo3 gene. FOXO3 is expressed throughout the body. It is phosphorylated and inactivated by the PI3K/AKT pathway. The phosphorylated and inactive form of FOXO3 is localized in the cytoplasm, but it is translocate into the nucleus after dephosphorylation and activation, where it exerts its transcription function. Our results corroborate those of [40] who also observed that treatment with Cbx reduced cerebral injury in rats exposed to transient focal

ischemia and reperfusion (I/R), and this was mediated by the activation of the PI3K/Akt pathways as well as by blocking the caspase 3 apoptosis pathway. This study examined the potential renoprotective effects of Carbenoxolone in cases of acute kidney injury (AKI) caused by renal ischemia-reperfusion injury (RIRI). Significantly, this therapeutic agent had not been previously investigated for their protective effects against acute kidney injury in this model. Our findings demonstrated substantial protective effects of Cbx, underscoring their potential as innovative therapeutic alternatives for alleviating ischemia-reperfusion-induced renal injury. This study is, to our knowledge, the first to assess and demonstrate the efficacy of Carbenoxolone in this model. These findings facilitate subsequent research into their mechanisms of action and their relevance in clinical.

EFFECT OF RIRI ON KIDNEY PARENCHYMA

Histological analysis revealed an increase in tissue damage after IRI, as shown by the presence of Cytoplasmic swelling and increased cytoplasmic eosinophilia, vacuolization, dilated renal tubules, glomerular alterations and hemorrhage, as well as a dilatation of the Bowman's capsule and a lack of brush boundaries. These changes had been in agreement with some other studies [41-43], that showed the same histopathological changes.

EFFECT OF CARBENOXOLONE ON KIDNEY PARENCHYMA

The findings demonstrated that Cbx pretreatment significantly ($P < 0.001$) reduced kidney injury severity compared to control and vehicle groups. The Cbx-treated group exhibited moderate structural changes, while the other groups showed severe damage. Notably, this is the first study to explore Cbx as a protective agent against renal ischemia-reperfusion injury (RIRI). Its antioxidant and anti-inflammatory properties likely contribute to its protective effects by reducing oxidative stress and inflammation—key drivers of RIRI-induced damage. These findings suggest Cbx as a potential therapeutic intervention for preserving renal tissue integrity and mitigating kidney injury. One of them [44] showed that the curcumin-treated group exhibited significant attenuation of these lesions, with only mild congestion and edema observed in the renal medulla. The renal tissue structure of the curcumin group was markedly improved compared to the RIRI group and resembled near-normal histology. The Paller scores, reflecting the severity of renal injury, were significantly higher in the RIRI group than in the sham group $P<0.01$. However, curcumin treatment significantly reduced these scores compared to the RIRI group $P<0.01$, highlighting its protective effects in mitigating renal tissue damage caused by ischemia-reperfusion injury. On the other hand [45] reported that in a transient cerebral ischemia model (30 min middle cerebral artery occlusion, 24 hr. reperfusion), CBX (25 mg/kg, intracerebroventricular) significantly reduced infarction size in rats by decreasing ROS production, thereby alleviating oxidative stress. CBX also inhibited astrocyte and microglia activation, reducing neuroinflammation and improving histopathological outcomes, including decreased apoptosis, preserved tissue architecture, and enhanced cell viability in the ischemic penumbra. Additionally, demonstrated that CBX supplementation provided Neuroprotection against Aβ 1-42 oligomer-induced hippocampal damage by improving neuronal viability.

CONCLUSIONS

Taken together, these results reinforce the potential of Carbenoxolone as a therapeutic agent in AKI management. Its ability to reduce kidney injury markers, suppress inflammation, restore antioxidant defenses, and modulate apoptotic pathways highlights its multifaceted protective effects. The translational significance of these findings underscores the need for further investigations to establish Cbx. as a viable medical intervention, ultimately improving patient outcomes in nephrology and critical care settings.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Munther Abosaooda
Collage of Pharmacy
The Islamic University, Najaf, Iraq
e-mail: munther.abosaooda@iunajaf.edu.iq

ORCID AND CONTRIBUTIONSHIP

Munther Abosaooda: 0000-0003-1755-6278 B C D E
Qayssar Joudah Fadheel: 0000-0003-3301-8357 A B C D E F

A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

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