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Autophagy and Oxidative Balance Mediate the Effect of Carvedilol and Glibenclamide in a Rat Model of Renal Ischemia-Reperfusion Injury

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Abstract

BACKGROUND: Reactive oxygen species and cytokines are the main players in the development of renal ischemia-reperfusion (I/R) injury.

AIM: The current study aimed to evaluate the effects of carvedilol and/or glibenclamide and the interaction between autophagy and oxidative stress.

METHODS: 50 male rats were divided into five groups: Control, IR injury (IRI), carvedilol pretreated, glibenclamide pretreated, and combined carvedilol and glibenclamide pretreated. Measurements of renal blood flow (RBF), creatinine clearance, serum blood urea nitrogen (BUN), histopathological, and immunohistochemical evaluation of autophagy marker Becl-1 in the rat kidney were performed. Beclin-1and light chain 3 (LC3) Mrna expression was detected by real time polymerase chain reaction.

RESULTS: IRI was associated with significant increases in BUN, tumor necrosis factor-alpha, nuclear factor κB, and histo (H) score value of Becl-1. However, there was a significant decrease in RBF, creatinine clearance, and glutathione peroxidase compared to the control group. There was significant increase in Beclin-1 and LC3 mRNA gene expression in carvedilol, glibenclamide, and combined treatment groups as compared to IRI and control groups. Combination of carvedilol and glibenclamide significantly restored IRI changes when compared with the other pretreated groups.

CONCLUSION: This study suggests that carvedilol and glibenclamide are promising reno-protective drugs to reduce renal injury induced by I/R through their antioxidant and autophagy stimulation.

Introduction

The kidney is one of the most sensitive organs susceptible to ischemic injury. Renal ischemia-reperfusion injury (IRI) is a common cause of acute renal failure, rejection of renal grafts, and renal cell death. It is commonly encountered in clinical situations, such as trauma, hypovolemic shock, and surgical procedures involving partial clamping of the renal vasculature such as partial nephrectomy or renal revascularization [1].

IRI includes a complex of inflammatory processes that involves the release of reactive oxygen species (ROS) and pro-inflammatory cytokines. Therefore, oxidative stress and inflammation are probably the key players in the development of renal dysfunction following renal IRI [2].

Carvedilol is a nonselective β-adrenergic antagonist, acting by blocking of the β arrestin signaling pathway of β adrenoreceptor. Furthermore, it acts as a selective alpha1-adrenergic antagonist, and exhibits additional ameliorating effects on oxidative stress and inflammation [3].

Glibenclamide is an ATP-sensitive potassium channel blocker, suggested to exhibit a reno-protective effect by inhibiting the production of inflammatory mediators through Sulfonylurea receptor 1 (Sur1) and the regulatory subunit of the K_{ATP} channel in ischemic tissue. Blocking the K_{ATP} channel is also claimed to suppress neutrophil migration and chemotaxis during the acute inflammatory response of renal IRI [4].

Autophagy is a physiological catabolic process that involves the lysosomal degradation of cytosolic components. Therefore, it is essential for cell homeostasis. Beclin-1 and microtubule-associated protein light chain 3 (LC3) are two pacemakers in the autophagic cascade [5].

After discerning the roles of ROS in renal IRI, several protective and therapeutic modalities have been assessed to overcome the severity of pathophysiological effects of ROS on the cellular

metabolism. In our experimental study, we aimed to evaluate the potential protective effect of carvedilol and glibenclamide alone or in combination on renal IRI, the possible underlying mechanisms and shed light on autophagy process.

Materials and Methods

The practical part of this study was carried out in the Department of Clinical Pharmacology, the Department of Clinical Physiology and the Department of Medical Biochemistry, Faculty of Medicine, Menoufia and Benha University, Egypt, the study was approved by the Medical Ethics Committee of Faculty of Medicine, Menoufia University under the number 3/2020PHAR5. All rats were treated according to the National Academies Press (NAP) Guide for the Care and Use of Laboratory Animals (eighth edition, NAP) [6].

Experimental animals

In this study, 50 adult male albino rats of Sprague Dawley strains, weighting 150–200 g, purchased from a local vendor were used. Rats were housed (5 per cage) in fully ventilated cages (70 × 70 × 60 cm). Standard water and food were allowed. Before starting experiments, all animals were acclimatized for 1 week to controlled conditions: Average temperature of 25 ± 2°C, relative humidity of 50%, and 12 h of light/dark cycles.

Rats were divided into the following groups:

1. Control group (10 rats): Rats received 1% DSMO by gastroesophageal lavage.
2. IRI group (10 rats): Rats received 1% DSMO by gastroesophageal lavage once daily 7 days before renal IRI operation as described below.
3. Carvedilol pretreated renal I/R injury (CRV + IRI) (10 rats): Rats received 2 mg/kg of carvedilol (Roche Pharmaceutical Company, Egypt), dissolved in 1% DSMO, by gastroesophageal lavage once daily for 7 days before renal IRI operation [7].
4. Glibenclamide pretreated renal I/R injury (G + IRI) (10 rats): Rats received 20 mg/kg of glibenclamide (Aventis Pharmaceutical Company, Egypt), dissolved in 1% DSMO, by gastroesophageal lavage once daily for 7 days before renal IRI operation [4].
5. Combined carvedilol and glibenclamide pretreated renal I/R injury (CRV + G + IRI) (10 rats): Rats received 2 mg/kg carvedilol and 20 mg/kg glibenclamide, dissolved in 1% DSMO, by gastroesophageal lavage once daily for 7 days before renal IRI operation.

Experimental designs

Induction of renal IRI

Under aseptic condition, rats were anesthetized with 2% pentobarbital sodium (50 mg/kg, i.p.). A midline laparotomy was performed in which the abdominal cavity was exposed, and rats were subjected to a right nephrectomy. Then, the left renal pedicle was clamped for 40 min using non traumatic artery clamp followed by reperfusion for 24 h [8].

Measurement of renal blood flow velocity (RBFV) and renal vascular resistance (RVR)

RBFV and RVR were measured using a bidirectional blood flow meter with FFT-analysis (HADECO, Japan). Each rat was anesthetized as the previous method, then, a midline laparotomy was made to expose the left renal artery. After setting the mode of pulsed blood flow meter (Doppler), the ultrasonic probe was pressed softly to the measured area at an angle of 40–50°. We wait for 5 s after hearing optimal sound without moving the probe then, the wave was freezed by pressing the freez key, RBFV and RVR were measured [9].

Measurements of creatinine clearance

The rats were housed individually in metabolic cages and 24 h urine samples were collected in graded tubes, during urine collection the animal received free access to water, but no food was given to avoid contamination. Creatinine clearance (ml/min) was calculated by using the formula: Creatinine clearance = U × V/P Where; U: Creatinine concentration in urine (mg/dl), V: Urine volume per minute (ml/min), and P: Creatinine concentration in plasma (mg/dl) [10].

Blood sample

Blood samples were collected under anesthesia through abdominal aorta, centrifuged and serum were separated for the measurement of blood urea nitrogen (BUN) and creatinine using the conventional colorimetric assay (Diamond diagnostic, Germany).

Preparation of kidney homogenate

After blood samples collection, a dry weight of 1 g of the left kidney was homogenized in 5 ml of cold phosphate buffered saline using an electronic tissue homogenizer (IKA-WERK, Ultra-Turrax, Germany) and the homogenate was centrifuged (10,000 rpm at 4°C for 15 min). The supernatant was frozen and stored at −80°C until used for the measurement of malondialdehyde (MDA) and glutathione peroxidase enzyme (GPX) by colorimetric assay, (Bio-diagnostic

company, Egypt). Tumor necrosis factor-alpha and nuclear factor κB (NF-κB) were determined by an ELISA kit (Assaypro LLC, Charles, MO, USA) and (MyBiosource, Inc. Southern California, San Diego, USA), respectively. Total RNA extraction by RNeasy extraction kit (Qiagen-USA).

Histopathology and immunohistochemistry (IHC)

Kidney IHC staining was performed using Beclin-1 rabbit polyclonal antibody (cat. no. YPA1333; Chongqing Biospes Co. Ltd, Chongqing, China) 100 μg/mL was used. It was enclosed in a single vial containing 0.1 ml of concentrated antibody and diluted before use with EDTA to 1: 200. IHC staining was performed using the streptavidin–biotin method (Universal Dako Cytomation LSAB2 system; DAKO, Glostrup, Denmark). Briefly, the primary antibody was applied on the slides and incubated overnight in humid chamber at room temperature. Bound antibody was detected using a modified labeled avidin–biotin reagent for 20 min and then washed with phosphate-buffered saline. The reaction was visualized by appropriate chromogen (diaminobenzidine, DAB) with Mayer's hematoxylin as a counterstain. The staining procedure included negative tissue control prepared by omitting the primary antibodies, and positive tissue control was fibroid tissue [11]. IHC interpretation of Beclin1 was assessed in all studied group; any cytoplasmic staining of Beclin 1 was considered positive. The distribution of positivity in the glomeruli or tubules was evaluated. Finally, The Histo score (H) was calculated with both the intensity of staining (mild, moderate, or marked) and the percentage of positivity as follows: 39 (% of strong intensity) + 29 (% of moderate intensity) + 19 (% of slight intensity). The H score ranges between 0 and 300 were divided into two categories: low (<150) and high (≥150) [12].

Detection of autophagy by quantitative real-time polymerase chain reaction (PCR) for Beclin-1 and LC3 genes

Total RNA was extracted from renal tissue by the RNeasy extraction kit (Qiagen-USA) according to the manufacturer's protocol. RNA concentration and purity were measured using nanodrop spectrophotometer (Biowave II Germany). Quantitative PCR was applied using the Ready-Mix PCR Reaction Mix kit (iTaq Universal SYBR Green One-Step Kit (Bio-Rad, USA). PCR thermal cycling conditions were: 10 min at 50ºC, 1 min at 95ºC then 40 cycles (15 s at 95ºC 30 s at 60ºC and 1 min at 55ºC) using Rotor gene real time PCR system and the related software for analysis and interpretation (Qiagen-S. Korea). GAPDH used as the reference gene for internal control. The PCR primers sequences are as follow, beclin-1-forward 5'-ACCGTGTCACCATCCAGGAA-3', beclin-1-reverse 5'-GAAGCTGTTGGCACTTTCTGT-3', LC3 forward primer(5'-TTCTTCCTCCTGGTGAATGG-3'),LC3reverse primer (5' GTCTCCTGCGAGGCATAAAC-3'), GAPDH-forward 5'-GAAATCCCATCACCATCTTCCAGG-3', GAPDH-reverse 5'-GAGCCCCAGCCTTCTCCATG-3'; and the expression level was calculated by using the comparative ($2^{-\Delta\Delta Ct}$) method. For more accuracy, the RT-PCR assays were performed in triplicate.

Statistical analysis

Differences between groups were determined by one-way analysis of variance and *post hoc* Tukey test using IBM SPSS 22 (SPSS Inc., Chicago. IL, USA) was used for analysis of data and bivariate correlation. The results were expressed as mean ± standard deviation (SD) of mean. $p \leq 0.05$ were considered statistically significant.

Results

RBFV and RVR

Figure 1 demonstrates RBFV in all the studied groups. The mean value of RBFV in IRI group was significantly lower than that in control group (2.97 ± 0.2 vs. 6.65 ± 0.4 cm/s) RBFV values in (CRV + IRI), (G + IRI), and (CRV + G + IRI) groups were significantly higher than that in IRI group (4.29 ± 0.19, 4.19 ± 0.18, 5.3 ± 0.1 cm/s, respectively) and significantly lower than that in control group. The RBFV value in the (CRV + G + IRI) group was significantly higher than those in the (CRV + IRI) group and in the (G + IRI) group. There was insignificant difference in the RBFV between (CRV + IRI) group and (G + IRI) group. Concerning RVR, the mean value in the IRI group was significantly higher than in the control group (1.5 ± 0.07 vs. 0.63 ± 0.04). Its values in the

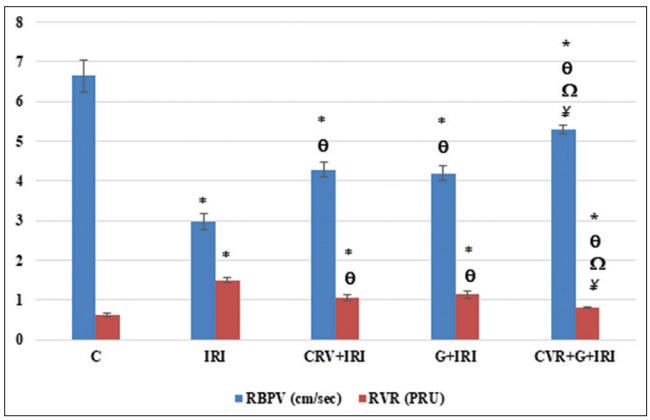

Figure 1: RBFV (cm/s) and RVR (PRU) in all studied groups. Results are expressed as mean ± SD (n = 10). Significance was considered when p < 0.05. The marks, Φ, Ω, and ¥ indicate that values are significantly different, when compared with the corresponding values of C, IRI, CRV + IRI, and G + IRI groups, respectively*

(CRV + IRI), (G + IRI) and (CRV +G + IRI) groups were significantly lower than those of the IRI group (1.06 ± 0.07, 1.14 ± 0.1, and 0.81 ± 0.03), respectively, and significantly higher than those of the control group. RVR value in the combined (CRV +G + IRI) group was significantly lower than of the (CRV + IRI), (G + IRI) groups. There was insignificant difference in the RVR between (CRV + IRI) and (G + IRI) groups.

BUN, serum creatinine, and creatinine clearance

Figure 2a demonstrates BUN in all studied groups. The mean value of BUN in IRI group was significantly higher than that in control group (204.84 ± 5.6 vs. 46.29 ± 0.78 mg/dl). BUN values in the (CRV + IRI), (G + IRI), and (CRV +G + IRI) groups were significantly lower than that in the IRI group (194.49 ± 1.4, 196.08 ± 0.92, and 77.01 ± 1.07 mg/dl), respectively, and significantly higher than that in control group. BUN in the (CRV + G + IRI) group was significantly lower than in the (CRV + IRI), and (G + IRI), groups. There was insignificant difference in the BUN in (CRV + IRI), and (G + IRI) group. Concerning serum creatinine (mg/dl) Figure 2b, the mean value of it in the IRI group was significantly higher than in the control group (1.16 ± 0.39 vs. 0.62 ± 0.22 mg/dl), creatinine values in the (CRV + IRI), (G + IRI), and (CRV + G + IRI) groups were significantly lower than in the IRI group (0.76 ± 0.005, 0.92 ± 0.006, and 0.67 ± 0.005 mg/dl), respectively, and significantly higher than in the control group. Its value in the (CRV + G + IRI) group was significantly lower than those in the (CRV + IRI) and (G + IRI), groups. Serum creatinine in the (CRV + IRI) was significantly lower than that in the (G + IRI) group. As regard to creatinine clearance (ml/min), the mean value in the IRI group was significantly lower than that in the control group (0.42 ± 0.1 vs. 1.4 ± 0.09 ml/min, its values in the (CRV+ IRI), (G+ IRI), and (CRV + G + IRI) groups were significantly higher than that in the IRI group (0.82 ± 0.04, 0.8 ± 0.04, and1.08 ± 0.11 ml/min), respectively, and significantly lower than that in the control group. Clearance value in (CRV + G + IRI) group was significantly higher than those in the (CRV + IRI) and (G+ IRI) groups. There was insignificant difference in the clearance between the (CRV + IRI) and (G + IRI) groups.

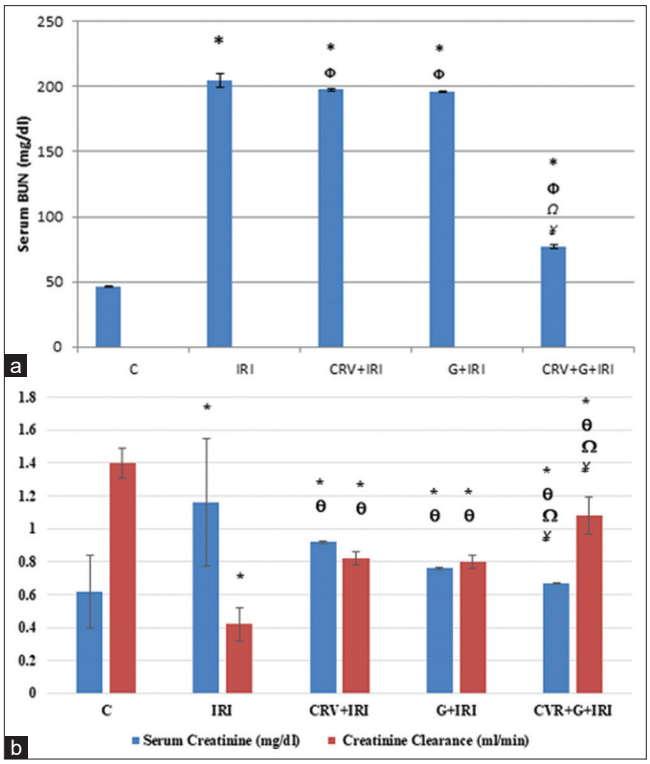

Figure 2: (a) Serum BUN (mg/dl) and (b) creatinine (mg/dl) and creatinine clearance (ml/min) in all studied groups. Results are expressed as mean ± SD (n = 10). Significance was considered when p < 0.05. The marks, Φ, Ω, and ¥ indicate that values are significantly different, when compared with the corresponding values of C, IRI, CRV + IRI, and G + IRI groups, respectively*

Renal oxidative stress markers (MDA and GPX)

Figure 3 shows that tissue MDA (nmol/g) in the IRI group was significantly higher than in the control group (94.67 ± 0.43 vs. 47.02 ± 0.75 nmol/g). The MDA values in the (CRV + IRI), (G + IRI), and (CRV + G + IRI) groups were significantly lower than that in the IRI group (59.7 ± 0.39, 55.8 ± 0.39, and 50.34 ± 0.39 nmol/g), respectively, and significantly higher than that in the control group. Its value in the (CRV + G + IRI) group was significantly lower than those in the (CRV + IRI) group and (G + IRI) group. MDA in the (CRV + IRI) group was significantly higher than that in (G + IRI) group. Regarding tissue GPX (u/mg), the mean value in IRI group was significantly lower than that in the control group (29.5 ± 2.2 vs. 56.39 ± 2.87 u/mg). Its values in the (CRV + IRI), (G + IRI), and (CRV + G + IRI) groups were significantly higher than in the IRI group (41.25 ± 1.2, 49.7 ± 0.9, and 54.9 ± 2.9 u/mg, respectively) and significantly lower than that in the control group. GPX

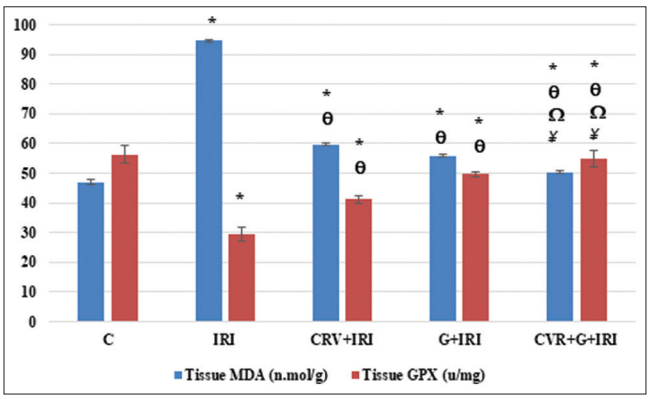

Figure 3: Tissue MDA (nmol/g) and GPX (u/mg) in all studied groups. Results are expressed as mean ± SD (n = 10). Significance was considered when p < 0.05. The marks, Φ, Ω, and ¥ indicate that values are significantly different, when compared with the corresponding values of C, IRI, CRV + IRI, and G + IRI groups, respectively*

value in the (CRV +G + IRI) group was significantly higher than those in the (CRV + IRI) and (G + IRI) groups. GPX in the (CRV + IRI) was significantly lower than in the (G + IRI) group.

Renal pro-inflammatory cytokines

Figure 4a illustrates tissue TNF-α. The mean value in the IRI group was significantly higher than that in the control group (69.5 ± 0.5 vs. 28.1 ± 0.49 pg/g, respectively; $p < 0.05$). Its values in the (CRV + IRI), (G + IRI), and (CRV + G + IRI) groups were significantly lower than that in the IRI group (32.9 ± 0.52, 37.1 ± 2.6, and 30.8 ± 0.5 pg/g), respectively, ($p < 0.05$) and significantly higher than that in the control group ($p < 0.05$). TNF-α value in the (CRV + G + IRI) group was significantly lower than those in the (CRV + IRI) and (G + IRI) groups. TNF-α in the (CRV + IRI) group was significantly lower than that in the (G + IRI) group ($p < 0.05$) concerning NF-κB. The mean value of tissue NF-κB in the IRI group was significantly higher than that in the control group (10.16 ± 0.34 vs. 1.7 ± 0.2 ng/g, respectively; $p < 0.05$). NF-κB values in the (CRV + IRI), (G + IRI), and (CRV +G + IRI) groups were significantly lower than that in the IRI group (5.81 ± 0.28, 6.75 ± 0.11, and 4.66 ± 0.18 ng/g), respectively, ($p < 0.05$) and significantly higher than that in the control group ($p < 0.05$). Its value in the (CRV +G + IRI) group was significantly lower than those in the (CRV + IRI), and (G + IRI), groups ($p < 0.05$). NF-κB in the (CRV + IRI) was significantly lower than that in the (G + IRI) group; ($p < 0.05$) Figure 4b.

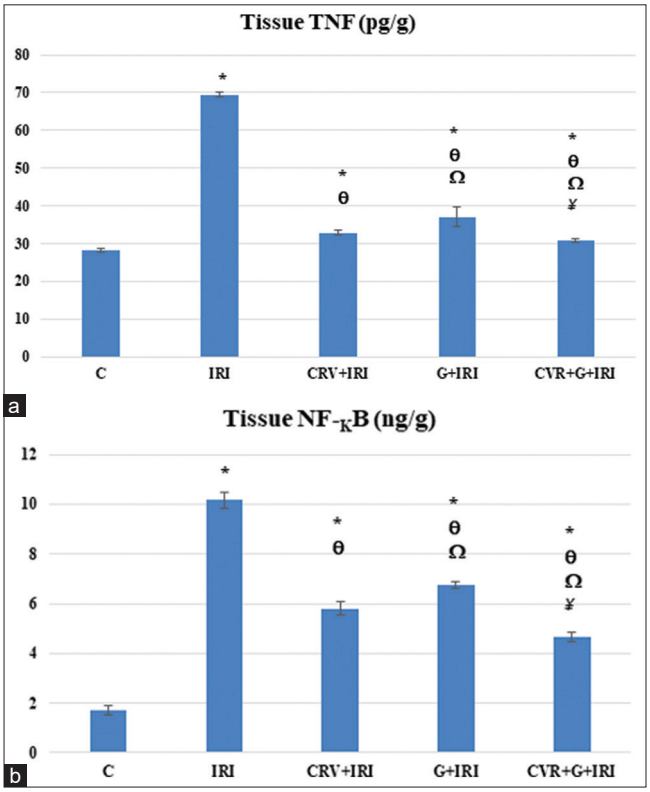

Figure 4: (a) Tissue TNF -α (pg/g) and (b) NF-κB (ng/g) in all studied groups. Results are expressed as mean ± SD (n = 10). Significance was considered when p < 0.05. The marks, Φ, Ω, and ¥ indicate that values are significantly different, when compared with the corresponding values of C, IRI, CRV +IRI, and G+IRI groups, respectively*

Histological and IHC results

Figure 5 demonstrates H&E-stained sections of rats' kidneys in all studied groups; The IRI group showed subtotal denuded tubular lining and the rest of lining showed degenerative vascular change. While section of the (CRV + IRI) group showed few renal tubules exhibited mild degree of degenerative changes in tubular epithelium. The histological changes in the (G + IRI), group showed many tubules exhibited moderate degree of degenerative changes in tubular epithelium. Combined treatment by both drugs in the (CRV +G + IRI) group showed renal tubules with unremarkable pathological changes.

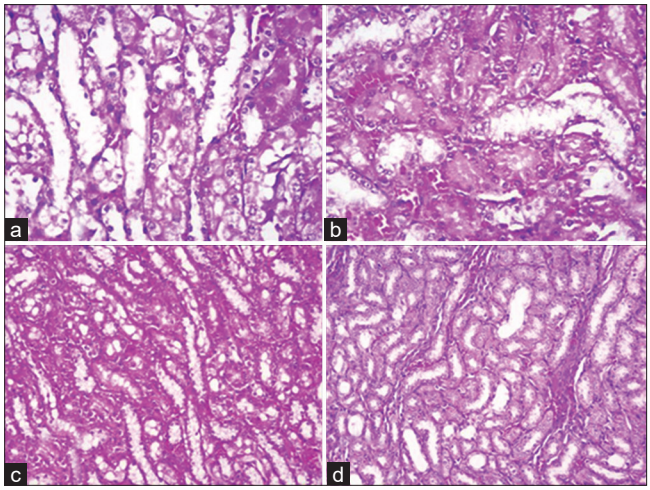

Figure 5: H&E-stained sections of kidneys in all studied group; (a) IRI group showed subtotal denuded tubular lining. The rest of lining showed sever degenerative vascular change. (b) CRV+ IRI group showed few renal tubules were exhibiting mild degree of degenerative changes in tubular epithelium. (c) G + IRI group showed many tubules exhibiting moderate degree of degenerative changes in tubular epithelium. (d) CRV+G+IRI group showed renal tubules with unremarkable pathological changes. H&E (X 400) for a and b; (X 200) for c and d

Figure 6 IHC staining of Beclin-1 in the kidneys, normal kidney showed mild patchy cytoplasmic expression of Beclin-1 in the glomeruli and absence of expression in the renal tubules. The IRI group showed mild diffuse cytoplasmic expression of Beclin-1 in the glomeruli and renal tubules. Whereas the (CRV + IRI) showed mild diffuse cytoplasmic expression of Beclin-1 in the glomeruli and moderated patchy cytoplasmic expression of Beclin-1 in the renal tubules but the (G + IRI), showed mild diffuse cytoplasmic expression of Beclin-1 in the glomeruli and the renal tubules. The (CRV + G + IRI) group showed marked patchy cytoplasmic expression of Beclin-1 in the glomeruli and renal tubules. Table 1 illustrates the H score of Beclin-1, in the glomeruli; there was an insignificant difference in all studied groups ($p > 0.05$). While H score of Beclin-1 in the tubules in the IRI was significantly

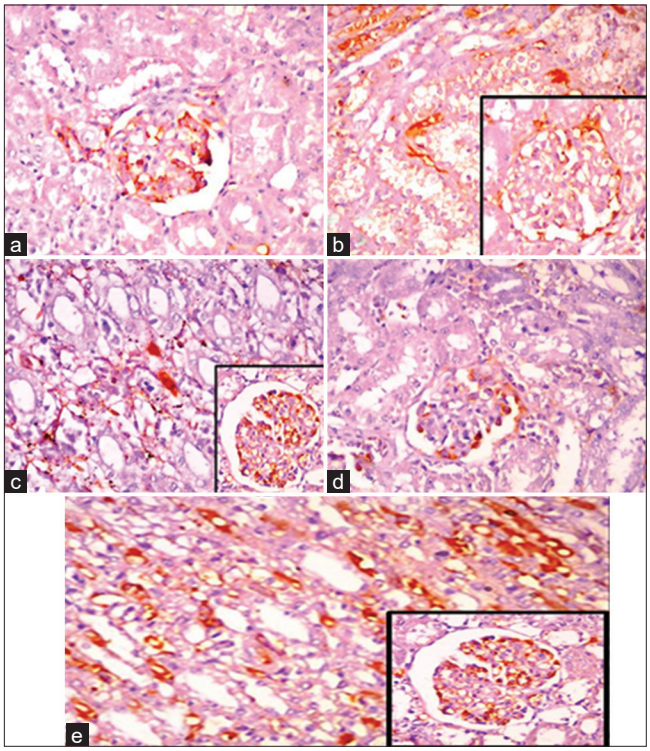

Figure 6: Beclin-1 IHC staining of kidneys in all studied group (a) normal kidney showed mild patchy cytoplasmic Beclin-1 expression in the glomeruli and absent expression in renal tubules. (b) IRI group showed mild diffuse cytoplasmic Beclin-1 expression in the glomeruli and renal tubules. (c) CRV + IRI group showed mild diffuse cytoplasmic Beclin-1 expression in the glomeruli and moderated patchy cytoplasmic Beclin-1 expression in the renal tubules. (d) G + IRI group, showed mild diffuse cytoplasmic Beclin-1 expression in the glomeruli and the renal tubules. (e) CRV +G + IRI group showed mild patchy cytoplasmic Beclin-1 expression in the glomeruli and marked patchy cytoplasmic Beclin-1 expression in the renal tubules. (Beclin-1 IHC x200 for a, b, c, d, and e)

higher than that in the control group ($p < 0.001$). Its values in the (CRV + IRI), (G + IRI), and (CRV +G + IRI) groups were significantly ($p < 0.001$) higher than that in the IRI. Its value in the (CRV + G + IRI) group was significantly ($p < 0.001$) higher than those in the (CRV + IRI) and (G + IRI) groups. H score in (CRV + IRI) was significantly higher than that in the (G + IRI) group ($p < 0.001$).

Table 1: H score of Beclin-1 in all studied groups

Parameters	Control	IRI	CRV + IRI	G + IRI	CRV + G + IRI
H score of Beclin-1 in Glomeruli					
Mean + SD	102.5 + 6.8	104.2 + 9.8	109.33 + 7.1	108 + 9.6	112.7 + 10
H score of Beclin-1 in tubules					
Mean + SD	0	52.5 + 11.2*	163.33 + 10.8*Φ	106 + 10.6*ΦΩ	230 + 22*ΦΩ¥

Results are expressed as mean±SD (n = 10). Significance was considered when P<0.05. The marks*, Φ, Ω, and ¥ indicate that values are significantly different, when compared with the corresponding values of C, IRI, CRV + IRI, and G + IRI groups, respectively.

Effect of carvedilol and glibenclamide on mRNA expression level of autophagy mediators, Beclin-1 and LC3

(Figure 7) Effect of carvedilol and glibenclamide on autophagy mediators, Beclin-1 and LC3 detected by quantitative RT-PCR show significant increase in mRNA expression of Beclin-1 and LC3 in the IRI group as compared to the control group. mRNA gene expression of Beclin-1 and LC3 in the (CAV + IRI), (G + IRI) and (CAV + G+IRI) groups is significantly increased as compared to the control group and IRI groups ($p < 0.05$). The relative gene expression values of Beclin-1 and LC3 in the (CRV +G + IRI) group were significantly higher than those in the (CRV + IRI), and (G + IRI), groups. Concerning the Beclin-1 gene expression, there was insignificant difference between the (CAV + IRI) and (G + IRI) groups.

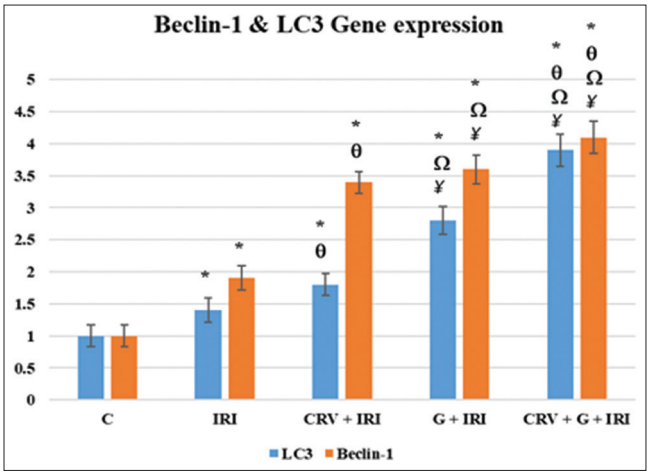

Figure 7: Effect of carvedilol and glibenclamide on gene expression of autophagy mediators, Beclin-1 and LC3 by real-time PCR show significant increase in mRNA expression of Beclin-1 and LC3 in CAV+IRI, G+IRI, and CAV + G + IRI groups as compared to control group and IRI groups ($p \leq 0.05$). Data were shown as mean ± SD

Discussion

Renal IRI is a major health problem and a common cause of acute kidney injury. In this study, IRI group exhibited a significantly lower RBF and glomerular filtration rate (GFR) when compared to the control group. These results agreed with Pechman *et al*. [13] who found that IRI could potentially increase the vascular resistance due to alteration of vasoactive factors and rarefaction of renal microvasculature. In addition, Gonullu *et al*. [14] suggested that sympathetic activation due to presynaptic noradrenaline release induced by renal stress resulted in reduced RBF and GFR. From the results of this work, an improvement in carvedilol group regarding both RBF and GFR was observed. This means that carvedilol has a Reno protective effect due to its ability to decrease RVR and improve renal hemodynamics by dilatation of the afferent and efferent arterioles [15]. Furthermore, glibenclamide group showed significant increase both RBF and GFR. This result was consistent with other studies [16] who explained this effect by decreasing RVR and inducing vasodilation of afferent and efferent arterioles. Interestingly, in our experiment, reperfusion

aggravates oxidative stress, evidenced by a significant elevation of the MDA level and reduction of the GPX level. This result agreed with Safari *et al*. [17] who found that reduction of renal GPX level and elevation of MDA was consequences of overproduction of free radicals leading to consumption of cellular antioxidants and failure of antioxidant defense mechanism to prevent oxidative tissue damage. Treatment with carvedilol or glibenclamide alone ameliorates oxidative stress after IR injury. These results were consistent with Zoroddu *et al*. [18] who reported that carvedilol could be a free radical scavenger by its free iron chelation, which would lead to inhibition of hydroxyl radical formation from both hydrogen peroxide and superoxide anion. The improvement in oxidative stress by glibenclamide is explained by reducing myeloperoxidase, and this might be mediated by the blockade of the membrane K_{ATP} channel [19].

Several studies revealed that the key factor in the development of IR injury was inflammation [26]. The IRI group showed a significant increase in pro-inflammatory markers of tissue TNF-α and NF-κB levels when compared to the control group. To support these findings, Gao *et al*. [20] found that renal tissues of IRI had a higher level of pro-inflammatory cytokines TNF-α and an inflammatory state triggered by activation of the inflammation-related signaling pathway as NF-κB pathway. The present study showed that pretreatment with carvedilol resulted in a significant decrease in TNF-α and NF-κB when compared to the IRI group. Consistent with the present results, Harhaj and Giam [21] reported that carvedilol reduced inflammation by restoring oxidative balance and produced its anti-inflammatory effects by downregulation of NF-κB pathway. Furthermore, glibenclamide showed significant decrease in pro-inflammatory mediators when compared to the IRI group. These results agreed with Bhaskar *et al*. [22] who stated that glibenclamide could prevent NADPH oxidase induced elevation in ROS and consequently inhibit NF-κB activation.

In this study, combined carvedilol and glibenclamide showed a significant improvement in all biochemical parameters measured when compared to the IRI and other treated groups, which was attributed to the synergistic effects of carvedilol and glibenclamide induced by their potent anti-inflammatory effects.

Immunohistochemical results showed a significant decrease in the H score of Beclin-1 in the IRI group, which was consistent with Zhang *et al*. [23], who found that IRI sensitized tubular cells to apoptosis by suppressing autophagy through down regulating the antiapoptotic Beclin-1. Furthermore, these results suggest that cravedilol exerts a protective effect on kidney by inhibiting the tubular cells apoptosis through upregulation of Beclin-1 resulting in autophagy stimulation. Hence, carvedilol showed a significant increase in H score of Beclin-1 when compared with the IRI group, which was consistent with [24] results. Furthermore, the glibenclamide group showed significant increase in H score of Beclin-1 when compared to the IRI group. To support this finding, Shi *et al*. [25] reported that glibenclamide could modulate autophagy and suppress tubular cell apoptosis through downregulation of Beclin-1 gene transcription. Furthermore, the combined group obviously showed significant increase of H score of Beclin-1 when compared with the other groups, which may be attributed to the potent stimulatory effect of autophagy activity of both carvedilol and glibenclamide.

Regarding renal function tests in IRI group, creatinine and BUN were significantly higher when compared to control group. The mechanism of IR-induced impaired renal function tests involves tubular renal injury, vascular injury, and inflammation [26]. Carvedilol demonstrated a significant improvement in renal function parameters in IRI rats, which is consistent with Akindele *et al*. [27] who reported better renal function and histological findings in the carvedilol treated group mediated by its antioxidant and vasodilator effect. Unlike the results of the present study, Küçük *et al*. [7] did not find a significant improvement in serum creatinine and BUN in the carvedilol group; however, carvedilol improved oxidative stress injury parameters such as MDA. Glibenclamide reduced creatinine and BUN in IRI rats. This finding can be explained by the fact that glibenclamide also has anti-inflammatory action by inhibiting the regulatory subunit of the K_{ATP} channel in ischemic tissue [28].

The present study showed significantly increased gene expression of Beclin-1 and LC3 in the CAV, G, and CAV+G groups as compared to the IRI and control groups. These results are consistent with the results of Zhang *et al*. [29] who showed that the expression of autophagy and anti-apoptotic-related proteins was significantly increased in the carvedilol group compared to that of the acute myocardial infarction group [29]. In addition, Su *et al*. [30] showed that glibenclamide induced an elevated protein level of the autophagy marker LC3-II in rat insulinoma cells. Hence, the present data suggest that combined glibenclamide + cravedilol-induced autophagy after I/R injury exerts a strong protective effect on the rat kidney. The highest expression of LC3 and Beclin-1, and increased number of autophagic vacuoles indicated that autophagy was better activated by combination of glibenclamide and cravedilol [31].

The present study showed that combined with carvedilol and glibenclamide showed significant improvement in renal function parameters better than each drug alone, this is due to the gained benefit from combined two different treatment mechanisms.

Conclusions

This study suggests that the administration of carvedilol or glibenclamide to rats with renal IRI has a

beneficial effect on the associated renal function and hemodynamics impairment, biochemical errors, and histological pictures. The combined administration of both carvedilol and glibenclamide improved renal functions, biochemical, and histological changes better than that of either of them alone. Hence, their combination before IRI is recommended.

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Data Availability

The data underpinning the work are not available

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