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Involvement of the Nrf-2/HO-1 Pathway in the Reno-protective Effect of Ambroxol Against Ischemia-reperfusion

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Abstract: Renal ischemic-reperfusion (I/R) injury is the primary leading cause of acute renal injury. Although ischemia contributes prominently to cellular death, reperfusion also results in more apoptosis in tubular cells that can precipitate an end-stage kidney disease. The anti-inflammatory and antioxidative activities of ambroxol have formerly been demonstrated. Objective: Evaluate the protective impact of ambroxol against renal injury induced by renal I/R, with emphasis on the impact of the Nrf-2/HO-1 pathway. Methods: Group I (Sham): Rats were daily administered distilled water (2 ml/Kg/p.o.), for seven consecutive days. They underwent all surgical procedures without clamping the renal arteries. Group II (I/R): Rats were treated as group I for seven days then subjected to renal ischemia (45 minutes) followed by 24-hours reperfusion period. Group III (Ambroxol+ I/R): Rats were daily administered ambroxol (70 mg/kg/p.o.), for seven days then exposed to renal ischemia and reperfusion as in group II. Renal function tests, renal oxidative stress state, renal expression of Nrf-2, as well as histopathological changes were assessed in all groups. Results: Renal I/R deteriorated kidney functions, triggered an oxidative stress status in renal tissues and increased the production of lipid peroxides, in addition to causing multiple histological changes. Pretreatment with ambroxol restores kidney function indicators and enhances antioxidant capacity. Moreover, ambroxol pretreatment dramatically ameliorated oxidative stress by raising the renal expression of Nrf-2 and the renal content of HO-1. Conclusion: Through its antioxidant properties, ambroxol could modulate the Nrf-2/HO-1 pathway, conferring a kidney impact against renal I/R.

Keywords: Ischemia-reperfusion; Oxidative stress; Ambroxol; Nrf-2; HO-1, rats.

1. INTRODUCTION

Renal ischemia-reperfusion (I/R) injury is a pathological condition that can develop in a diversity of clinical situations, such as shock, sepsis, organ transplant, and vascular surgery. It is associated with a high mortality rate and rapid kidney impairment [1,2].

Even though the pathophysiology of I/R injury in the kidney is very complex, a number of pathogenic processes are thought to be involved, including the generation of reactive oxygen species (ROS) and the oxidative stress they cause, intracellular calcium accumulation, mitochondrial uncoupling, and inflammatory immune response [3].

During the ischemic phase, reduced generation of adenosine triphosphate (ATP) leads to acidosis, cytoskeleton disintegration, lysosomal enzyme leakage, and suppression activity of the trans-membrane Na^+/K^+ ATPase, with consequent cellular edema and Ca^{2+} overloading, which is responsible for the generation of ROS [4-6]. Restoring blood flow during reperfusion encourages further Ca^{2+} overloading, ultimately leading to calpain activation and cell death [6]. Furthermore, the return of normoxia results in a robust generation of ROS that damage the cytoskeleton, DNA, and cell membranes [7]. Additionally, dysregulated Ca^{2+}causes opening of the transition pore in mitochondria and the release of chemicals such as succinate, cytochrome C, and mitochondrial DNA. The released substances have the ability to cause

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necrosis and apoptosis in cells, in addition to their potential role as danger- or damage-associated molecular patterns, activating both the innate and the acquired immune systems [8-10].

Nuclear factor erythroid 2 related factor-2, or Nrf-2, is a principal transcriptional regulator of the body's resistance mechanisms against oxidative damage [11]. Normal conditions cause Nrf-2 to be continually sequestered by its negative regulatory protein, Kelch like ECH associated protein 1, Keap1, and to be destroyed by the proteasome-ubiquitin pathway [12]. However, Nrf-2 separates from Keap1 and moves to the nucleus in response to redox imbalance. Stabilized Nrf-2 proteins connect with antioxidant response elements in nuclei and trigger the production of target genes for antioxidant enzymes including hemeoxygenase-1 (HO-1) [13]. Of note, Nrf-2 has been anticipated to play a vital role in the renal I/R injury pathogenesis by protecting the body against oxidative stress [14,15].

Hemeoxygenase-1 is a vital endogenous antioxidant and a crucial component of the immune response. Triggered by Nrf-2, HO-1, together with products of heme degradation (Fe, CO, and biliverdin), can scavenge singlet oxygen, hydroxyl radicals, and superoxide anions, thus prohibiting excessive oxidation of cellular lipids and proteins. They also have a beneficial influence on inflammatory and apoptotic processes [16].

Ambroxol "2 amino- 3,5 dibromo- N-[trans-4 hydroxycyclohexyl]benzylamine" has been commonly used since 1978 as an over the counter mucoactive medication for treatment of multiple respiratory illnesses [17]. The ability of ambroxol to stimulate the surfactant synthesis by pneumocytes underlies its properties as an effective mucokinetic and secretagogue agent [17]. Along with the antioxidant effects of the released surfactant, ambroxol itself exerts antioxidant properties via direct scavenging of ROS [17,18]. Additionally, ambroxol inhibits leukocytes from secreting inflammatory mediators, hence acquiring anti-inflammatory properties [19,20]. Recently, it has been demonstrated that ambroxol's anti-inflammatory and antioxidant characteristics could modify the progression of acetic acid induced ulcerative colitis in rats [18]. Moreover, the inhibition of Na channels has been suggested as a mechanism of ambroxol in ameliorating different types of pain in preclinical and clinical studies [21-23].

Regarding ischemic injury, ambroxol with α-lipoic acid contributed to reducing neuronal damage in organoid hippocampal cultures subjected to hypoxia and glucose deprivation and in gerbils exposed to cerebral ischemia [24]. The ability of ambroxol to exert hepatoprotective effect in model of hepatic I/R was also reported. Up-regulating the intra-cellular antioxidant and anti-apoptotic signalings have been proposed as a potential mechanism underlying protection against ischemia-related hepatic damage [25].

Though ambroxol influence on renal I/R-prompted pro-inflammatory cytokines upsurge and histopathological changes has been recently investigated [26], the role of ambroxol on different pathophysiological mechanisms that accompany renal I/R injury is a subject that needs to be explored. Thus, in our current study, we intended to give more insight into the preventive effect of ambroxol on renal I/R injury in rats. We demonstrated that ambroxol pretreatment inhibits renal I/R-induced kidney function deterioration, oxidative stress, and histopathological changes, an effect that is strongly related to the up-regulation of the Nrf-2/HO-1 pathway.

2. METHODS

2.1. Laboratory animals

Twenty-four adult male Albino rats (200-250 g) were utilized in the study. All rats were housed in stainless steel cages (four rats/ cage), with temperature maintained at 22 ± 2 °C and provided freely with rodent chow pellets and water. The study was conducted according to the Ethics Committee of Faculty of Pharmacy for Girls, Al-Azhar University, Egypt (# 294/2020).

2.2. Drugs and other chemical substances

Ambroxol was procured from GSK pharma (Giza, Egypt), as ambroxol hydrochloride in the form of white powder and was diluted to a final concentration of 3.5% using distilled water (2 ml). Sodium pentobarbitone and all other chemicals, solvents, and reagents used were highly analytical and obtained from Sigma Aldrich (St Louis, USA).

2.3. Inducement of renal ischemia-reperfusion

On the 7th day of ambroxol treatment and after an overnight fasting with unrestricted access to water, animals were anesthetized using sodium pentobarbitone (60 mg/kg, i.p.) [27]. A median abdominal incision was then made on all rats, and both right and left renal pedicles were surgically removed and blocked with vascular clamps for 45 minutes resulted in ischemia. After 45-minutes blockage period, the clamps were taken away and the abdominal incision was sutured into two layers. To prevent hypothermia, the animals were kept warm during the whole operation using a heating lamp [28].

2.4. Experimental design

After seven days of accommodation, 24 rats were randomly allocated into three groups (eight animals / group) as follows; group I (Sham): Rats were daily treated with distilled water (2 ml/Kg/p.o.), for seven consecutive days. They underwent all surgical procedures without clamping the renal arteries. Group II (I/R): Rats were treated as group I for seven days then subjected to renal ischemia for a 45-minutes period followed by 24 hours of reperfusion [28]. Group III (Ambroxol + I/R): Rats were daily administered ambroxol (70 mg/kg/p.o.), for seven consecutive days [29,30], then exposed to renal ischemia and reperfusion as in group II.

2.5. Samples preparation

After the reperfusion period, all animals were sacrificed, then blood samples were centrifuged for 15 min and serum was obtained and stored at -80ºC until analysis. In parallel, kidneys were directly excised from six rats in each group, rinsed with ice cold 0.9 % saline, and stored at −80°C for subsequent biochemical and western blotting analysis. Likewise, kidneys were taken from two animals in each group and preserved in buffered formalin (10 %) for subsequent pathological analysis.

2.6. Renal function biomarkers assessment

According to the procedures indicated by the appropriate spectrocolorimetric assay kits (Biodiagnostic Co., Cairo, Egypt), the blood urea nitrogen (BUN, **Catalog #** UR 2110) and serum creatinine (**Catalog #** CR 1251) were assessed respectively.

2.7. Determination of lipid peroxide formation and the total antioxidant capacity

As stated by the manufacturer's instructions, the lipid peroxidation, evaluated as malondialdehyde (MDA) content and the total antioxidant capacity (TAC) in renal homogenates were detected spectrophotometrically using the corresponding commercial kits (Biodiagnostic Co., Cairo, Egypt, **Catalog #**MD 2528 and **Catalog #**TA 2512, respectively).

2.8. Determination of renal HO-1 content

Renal content of HO-1 was detected by the enzyme linked immunosorbent assay using corresponding commercial kit (**Catalog #** MBS764989, My Biosource, San Diego, USA).

2.9. Western blot determination of renal Nrf-2 protein expression

Western blot analysis was used to measure the renal protein expression of Nrf-2 using the procedure described previously by **Mounieb** [31]. Total protein extraction from kidney homogenates was performed using ice-cold RIPA lysis buffer (Santa Cruz Biotechnology, California, USA), and measured with the Bradford Protein Assay Kit (**Catalog #** 23200, Thermo Fisher Scientific, USA). Protein extract samples were denatured using the sample buffer of Laemmli, separated by electrophoresis using 10 % sodium dodecyl sulphate-polyacrylamide gel, and transferred to a PVDF membrane. The membranes were then blocked in TBS-T with 3 % BSA and probed with the appropriate primary antibody for Nrf-2 (**Catalog #** ab137550, Abcam Cambridge, UK). Then, β-actin antibody (**Catalog #** A5316, Sigma-Aldrich, St Louis, USA) was added and kept at 4 °C for 1 h, followed by an addition of the secondary antibody (linked to HRP). Signals were identified using the Enhanced chemiluminescence kit, as recommended by the manufacturer (Beyotime, Shanghai, China).

2.10. Histopathological assessment

Renal tissues were preserved for a full day in 10% buffered formalin. Subsequently, they were dehydrated using various alcohol grades, cleaned using xylene, and then imbedded in paraffin wax. The 4-micron paraffin cuts were stained with the two dyes, hematoxylin and eosin (H and E). A pathologist used a light microscope to examine the cells [32].

2.11. Statistical analysis

The data were statistically analyzed with one way analysis of variance (ANOVA) and Tukey as a post hoc test. The data were expressed as the mean ± SD of six animals. The correlation between some variables was assessed using Pearson's correlation test. P-values below 0.05 were considered as indication of statistically significant differences between the situations under comparison. Data analysis and graphs sketching were achieved using Graph Pad Prism (version 5).

3. RESULTS

3.1. Ambroxol conserves the renal functions after renal I/R

Occlusion of the renal artery for 45 minutes followed by 24-hours of reperfusion significantly increased the serum level of BUN and creatinine by 102% and 575%, respectively, relative to the sham group, while pretreatment of I/R-challenged rats

with ambroxol significantly lessened the serum levels of BUN and creatinine by 34.3% and 66.3%, respectively, compared with the I/R group, as shown in **Figure 1 (A &B, respectively).**

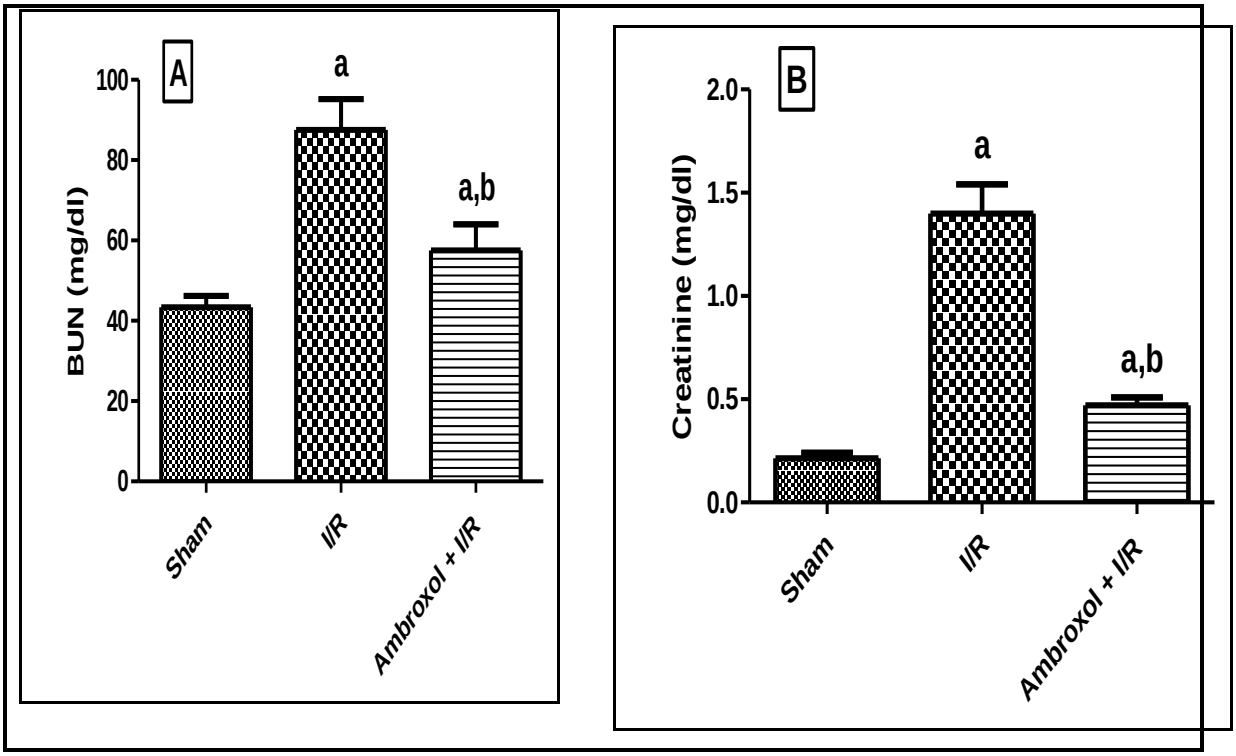

Figure 1. Effect of ambroxol pretreatment on kidney function markers [A, serum levels of blood urea nitrogen (BUN) and B, serum levels of creatinine] after renal ischemia-reperfusion in rats. Data was recorded as the mean ± the standard deviation (n = six rats per group). BUN, blood urea nitrogen; I/R, ischemia-reperfusion. a or b, Statistically significant from the sham or the I/R group, respectively at P less than 0.05.

3.2. Ambroxol mitigates the oxidative stress induced in renal tissues following renal I/R

Occlusion of the bilateral renal arteries in rats for 45 minutes followed by reperfusion for 24-hours induced a redox imbalance in renal tissues. The imbalance was determined by assessing MDA content and TAC.

Renal I/R significantly increased MDA content in renal tissues by 289.8%, in comparison to sham group, while ambroxol administration at a dose 70 mg/kg before renal I/R significantly decreased MDA content in kidney by 52.5% compared to I/R group **(Figure 2)**.

On the other hand, renal I/R significantly decreased TAC in renal tissues by 71.7 %, as amounted to the sham group, while pre-administration of ambroxol significantly increased the renal TAC by 124.5%, as compared to I/R group **(Figure 3)**.

3.3. Ambroxol attenuates renal impairment following renal I/R via up-regulating the Nrf-2/HO-1 pathway

As demonstrated in **Figure 4**, 45-minutes period of renal artery occlusion followed by a 24-hours period of reperfusion induced a significant decline in renal Nrf-2 expression by 64.7%, as compared to the sham group. When ambroxol was administered prior to renal I/R, renal Nrf-2 protein expression was significantly increased by 116.7%, as related to the I/R-challenged group.

As well, the renal content of HO-1 was significantly decreased by 70.7% by 45-minutes period of renal artery occlusion and 24-hours period of reperfusion, as equated to the sham group. Pre-dministration of ambroxol before renal I/R significantly enhanced the renal HO-1 content by 169%, as compared to the group subjected to I/R **(Figure 5)**.

3.4. Effect of ambroxol on the statistical correlation between the renal expression of Nrf-2 and the renal contents of HO-1, TAC, and MDA

Significant positive correlations were found between the renal expression of Nrf-2 and the renal contents of HO-1 (Pearson r = 0.9605, $P < 0.05$, **Figure 6A**), and TAC (Pearson r = 0.9547, $P < 0.05$, **Figure 6B**). On the other hand, a significant negative correlation was detected concerning the renal expression of Nrf-2 and the renal content of MDA (Pearson r = - 0.9740, $P < 0.05$, **Figure 6C**).

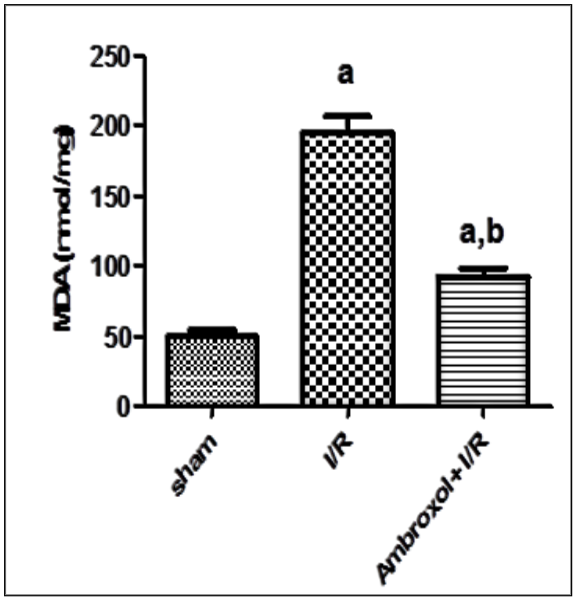

Figure 2. Effect of ambroxol pretreatment on renal content of malondialdehyde after renal ischemia-reperfusion in rats. Data was recorded as the mean ± the standard deviation (n = six rats per group). I/R, ischemia-reperfusion; MDA, malondialdehyde. a or b, Statistically significant from the sham or the I/R group, respectively at P less than 0.05.

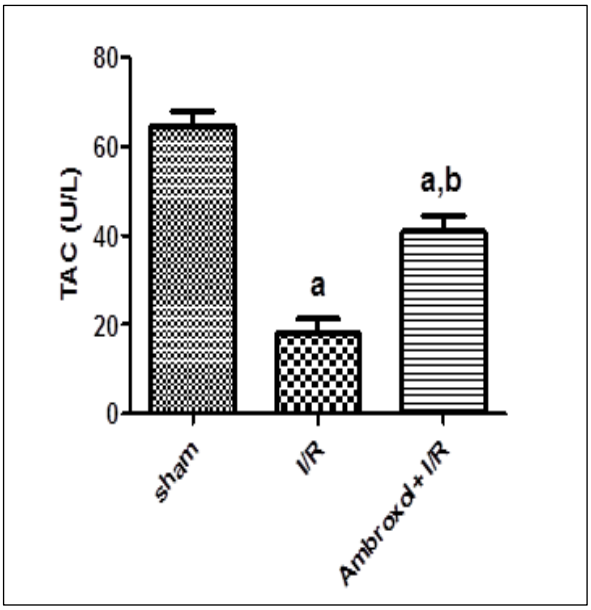

Figure 3. Effect of ambroxol pretreatment on renal total antioxidant capacity after renal ischemia-reperfusion in rats. Data was recorded as the mean ± the standard deviation (n = six rats per group). I/R, ischemia-reperfusion; TAC, total antioxidant capacity. a or b, Statistically significant from the sham or the I/R group, respectively at P less than 0.05.

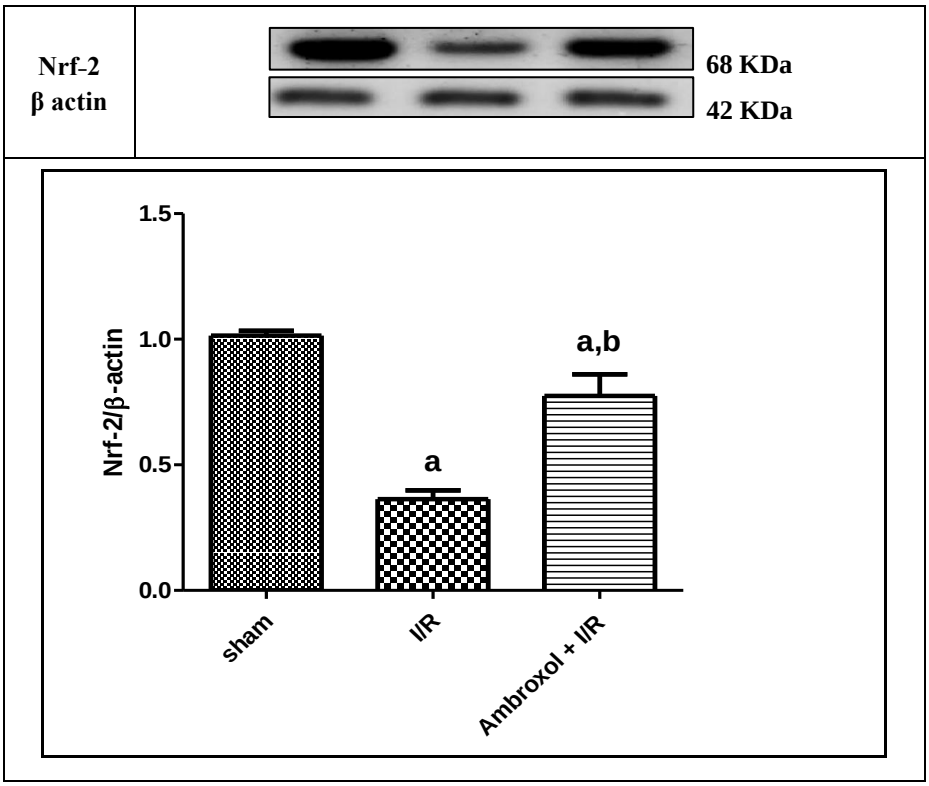

Figure 4. Effect of ambroxol pretreatment on renal expression of the nuclear factor erythroid 2-related factor 2 after renal ischemia-reperfusion in rats. Data was recorded as the mean ± the standard deviation (n = six rats per group). I/R, ischemia-reperfusion; Nrf-2, nuclear factor erythroid 2-related factor-2. a or b, Statistically significant from the sham or the I/R group, respectively at P less than 0.05.

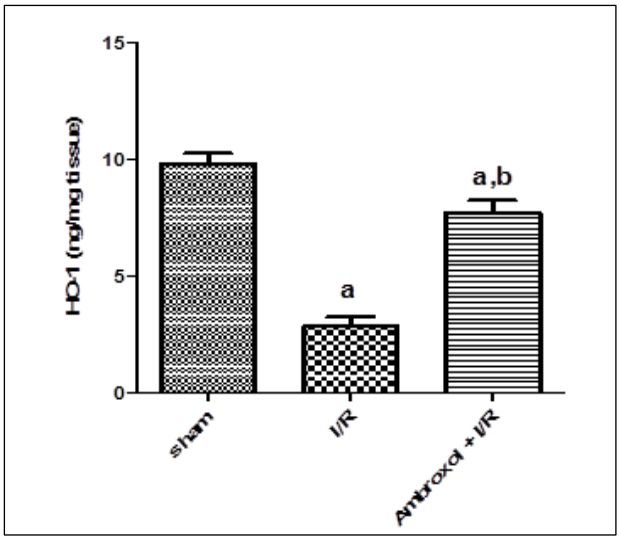

Figure 5. Effect of ambroxol pretreatment on renal content of hemeoxygenase-1 after renal ischemia-reperfusion in rats. Data was recorded as the mean ± the standard deviation (n = six rats per group). HO-1, hemeoxygenase-1; I/R, ischemia-reperfusion. a or b, Statistically significant from the sham or the I/R group, respectively at P less than 0.05.

3.5. Alterations in histopathology of renal tissues

Sections of kidney taken from the sham group stained with H and E demonstrated normal tubule ***(t)*** and glomeruli ***(g)*** histological structures at the cortex. Conversely, a 24-hours period of reperfusion after a 45-minute renal artery blockage caused many alterations in the kidney tissues. Nuclear pyknosis ***(p)*** and degeneration ***(d)*** in the epithelia lining the cortical tubules were observed in conjunction with swelled and vacuolated ***(v)*** endothelium lining the glomerular tufts. At the corticomedullary section, focal hemorrhages ***(h)*** were seen between the deteriorated tubules. At the corticomedullary region, tubular cystic dilatation ***(c)*** with flattened lining epithelium was also observed. Pre-treatment with ambroxol was found to ameliorate these changes. The cortical section in ambroxol treated group showed blood vessel ***(bv)*** congestion along with swelling and degeneration ***(d)*** in tubules' lining epithelium. Nuclear pyknosis ***(p)*** was also detected in the tubular epithelial lining at the corticomedullary portion **(Figure 7)**. Semi-quantitative histopathological scoring of the kidney slides is denoted in **Table 1**.

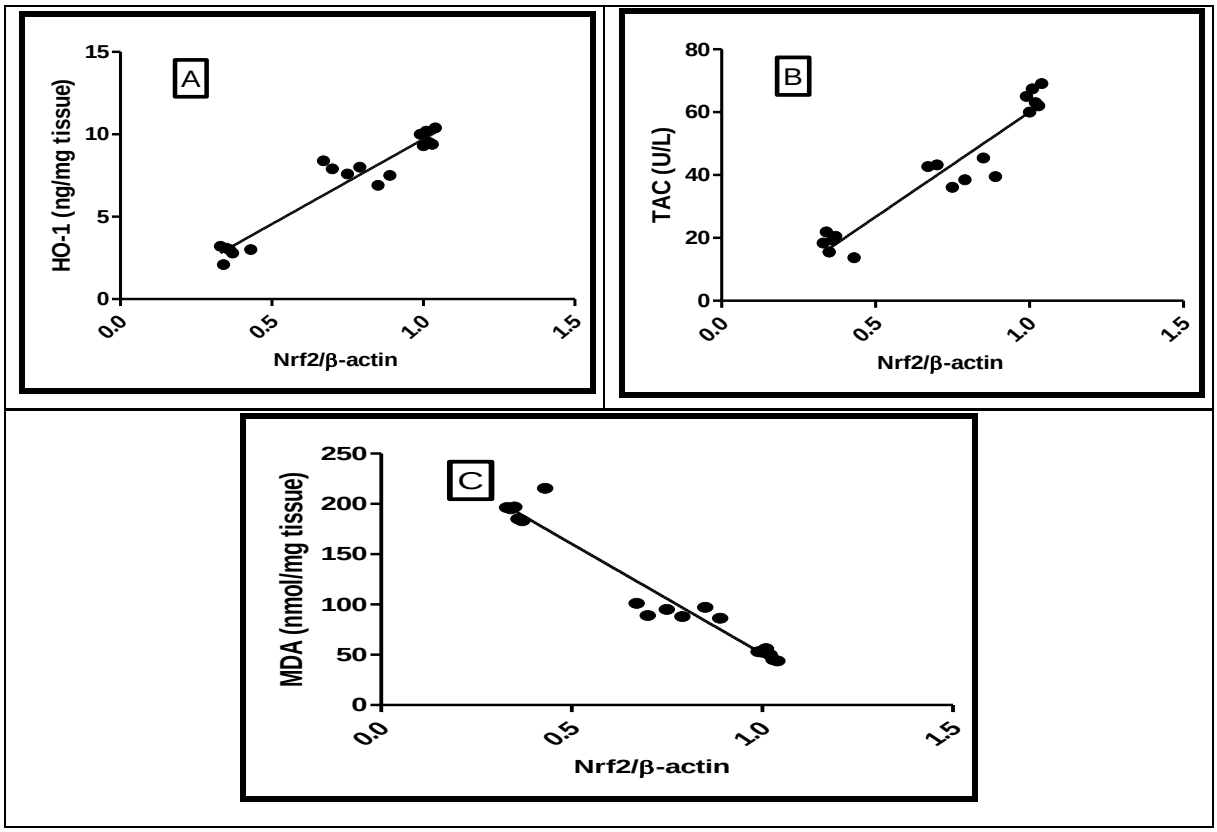

Figure 6. Correlation between the renal expression of the nuclear factor erythroid 2-related factor-2 (Nrf-2) and the renal contents of hemeoxygenase-1 (HO-1, A), the total antioxidant capacity (TAC, B), and malondialdehyde (MDA, C), in sham, ischemia-reperfusion (I/R, 45-minutes period of renal ischemia followed by 24 hours of reperfusion) and ambroxol (70 ml/Kg/p.o., daily for 7 days) + I/R groups.

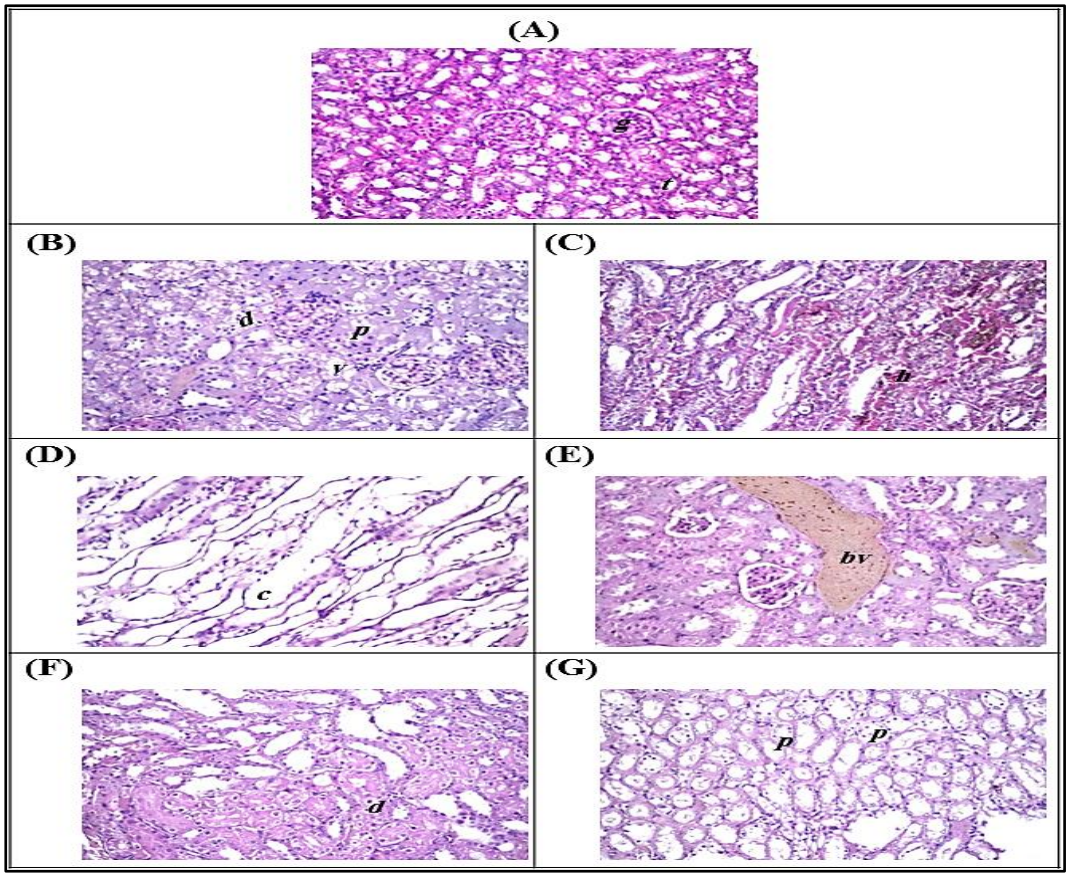

Figure 7. Representative photomicrographs of H- and E-stained kidney sections in renal I/R and/or ambroxol pretreated rats (x40). **(A):** normal histological architecture of the kidney tissue in sham group, **(B):** nuclear pyknosis ***(p)*** and degeneration ***(d)*** in the lining epithelium in renal I/R group, **(C):** focal hemorrhages ***(h)*** were noticed at corticomedullary portion in between the degenerated tubules in renal I/R group, **(D):** tubular cystic dilatation ***(c)*** with flattened lining epithelium associated with swelled and vacuolated ***(v)*** endothelium that lines the glomerular tufts in renal I/R group, **(E):** congestion in the blood vessels ***(bv)*** in cortical portion in rats pretreated with ambroxol, **(F):** swelling and degeneration ***(d)*** in epithelial lining in rats pretreated with ambroxol and **(G):** Nuclear pyknosis ***(p)*** and degeneration were detected in the lining tubular epithelium at the corticomedullary portion in rats treated with ambroxol before renal I/R.

Table 1. Semi-quantitative histological scoring of renal sections obtained from Sham, I/R (ischemia-reperfusion), and Ambroxol + I/R groups.

Group / **Histopathological alterations**	**Sham**	**I/R**	**Ambroxol + I/R**
Coagulative necrosis in the lining tubular epithelium	-	+++	++
Cast formation in tubular lumen	-	+++	-
Focal hemorrhage	-	+	-

4. DISCUSSION

The pathogenesis of renal I/R injury emerges as a complicated series of biochemical and structural incidents that contribute to tubular damage [33,34]. Over time, renal I/R-induced acute kidney injury can increase extracellular matrix deposition and fibroblast proliferation, ultimately progressing to end-stage kidney failure [6,35]. Although ischemic injury is a primary contributor to cellular death, reperfusion in itself produces more harm, resulting in more apoptosis in renal tubular cells [2]. From a molecular point of view, oxidative stress, ROS generation and lipid peroxidation are highly documented as critical in the development of renal I/R injury. Therefore, exploring interventions that supress these events can be a valuable approach to this issue [3].

In the existing study, we demonstrated the reno-protective impact of ambroxol, a non-prescription mucolytic agent used in treating respiratory diseases, in a rat model of renal I/R

injury. Ambroxol treatment significantly attenuated the I/R-induced renal functional and structural damage. We further demonstrated that the ambroxol antioxidative effect via Nrf-2/HO-1 pathway up-regulation is a main contributor to its protection against renal I/R injury.

Firstly, serum biochemical tests performed following a 45-minutes occlusion of the bilateral renal arteries and a reperfusion for 24 hours showed a significant rise in BUN and creatinine values, which aligns with former studies that implied significant upsurges in kidney function biomarkers as indication for renal damage and impaired glomerular filtration rate after renal I/R [36-38]. Pre-treatment with ambroxol at a dose equal 70 mg/kg resulted in a decrease in BUN and creatinine levels. This is consistent with previous investigations demonstrating the efficiency of ambroxol in normalizing aberrant kidney function in experimentally produced intestinal I/R, cisplatin-induced nephrotoxicity, and in children undergoing cardiopulmonary bypass [29,30,39].

The histological evaluation of renal tissues confirms the current biochemical findings. The kidneys of the untreated renal I/R group showed tubular cell enlargement, vacuolization, pyknotic nuclei, and cystic dilatation. Focal haemorrhages were also found between the deteriorated tubules. These alterations revealed widespread damage at the cortical and corticomedullary portions and are congruent with the research of Zhang et al. and Nezamoleslami et al. [40,41]. However, the pathological semi-quantitative score of renal injury indicated that tubular damage and focal haemorrhages were obviously attenuated by pre-treatment with ambroxol, verifying the cytoprotective potential of ambroxol [29,30].

Accumulating evidence suggests oxidative stress as a critical stage in the initiation and progression of renal I/R damage [42]. ROS are produced during renal ischemia, but the amount is substantially higher during the reperfusion phase, resulting in lipid oxidation and intensifying the damage [2,43]. MDA is a well-known secondary product of lipid peroxidation and the most widely used biomarker of oxidative damage to cells and tissues [44], while TAC is widely used to examine the antioxidant state of biologic materials and can determine the antioxidant reaction to free radicals provoked during certain disorders [45].

In the current study, MDA levels increased significantly while TAC levels decreased significantly in kidney tissues, showing that renal oxidative stress was the predominant cause of renal I/R. These results are in agreement with previous studies showed a substantial increase in oxidative stress markers in the kidney after renal I/R injury [46-48].

Remarkably, ambroxol administration had positive effects on kidney tissue biomarkers of oxidative stress, as seen by the decrease in MDA and the replenishment of TAC. These findings are consistent with other researches that have documented ambroxol's antioxidant properties [18,25,30].

The transcription factor, Nrf-2 is assumed to play a vital role in protection of many cells opposing oxidative stress by maintaining the antioxidant/oxidant homeostasis. Catalase, superoxide dismutase, HO-1, and others are among the cytoprotective genes whose transcription is directly regulated by Nrf-2 [13]. Notably, various scientific investigations have reported the protective impact of Nrf-2 pathway's activation against I/R injury in various organs [28,49,50].

According to the results of our study, oxidative stress caused by renal I/R significantly reduces the expression of Nrf-2 and the content of its downstream conjugating enzyme HO-1 in renal tissues. In line, several studies have reported the effectiveness of Nrf-2/HO-1 activation in alleviating renal I/R injury [51]. Indeed, knocking out or inhibition of Nrf-2 enhances susceptibility of kidney tissues to ischemic injury [52-54]. In addition, current results showed that ambroxol pre-treatment boosted the Nrf-2 activity, enhanced its expression along with its downstream gene; HO-1, hence rescuing animals from the kidney damage induced by renal I/R. Moreover, results of statistical correlation study revealed significant positive correlations between renal expression of Nrf-2 and renal HO-1 content and the TAC, and a negative correlation between the renal lipid peroxidation and Nrf-2 expression. These results add more proof to the association of antioxidant properties of ambroxol in its renoprotective effect against renal I/R and are consistent with Cavalu et al. and Bishr et al. [18,30].

5. CONCLUSIONS

The main findings of this work reveal the contribution of the Nrf-2/HO-1 pathway in the antioxidant potential of ambroxol against renal damage induced by renal I/R, thus affording a theoretical foundation for the clinical implementation of ambroxol in the prevention of ischemic kidney disease. However, other molecular mechanisms that may be regulated with ambroxol to relieve renal I/R injury necessitate further investigation.

Supplementary Materials:

Funding: No specific grant was given for this research by public, private, or nonprofit funding organizations.

Conflicts of Interest: There is no conflict of interest declared by the authors.

Ethical Statement: This study was executed with the approval of the Ethics Committee of Faculty of Pharmacy (Girls), Al-Azhar University (# 294/2020) following the standards of Principles of Laboratory Animal Care (NIH Publications # 85-23, reviewed 2011).

Author Contribution: HF conducted the whole experiment, gathered the data, carried out the graphical and statistical analyses, and drafted the manuscript. SA participated in conceptualization the research idea and the execution of the experiment, supervised the analyses of data and writing and revised the whole manuscript. AA conceptualized the research's idea, assisted in overcoming most of the challenges including training the practical techniques then thoroughly reviewed and revised the manuscript as a whole.

List of Abbreviations: AKI, acute kidney injury; ANOVA, analysis of variance; ATP, adenosine triphosphate; BUN, blood urea nitrogen; H and E, hematoxylin and eosin; HO-1, heme oxygenase-1; I/R, ischemia-reperfusion; Keap 1, Kelch-like ECH-associated protein 1; MDA, malondialdehyde; Nrf-2, Nuclear factor erythroid 2 related factor-2; ROS, reactive oxygen species; TAC, total antioxidant capacity.

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