Biomolecules and Biomedicine
ISSN: 2831-0896 (Print) | ISSN: 2831-090X (Online)
Journal Impact Factor® (2024): 2.2
CiteScore® (2024): 5.2
www.biomolbiomed.com | blog.bjbms.org

RESEARCH ARTICLE

Sirinyildiz et al: Arbutin in renal IRI

Arbutin as a potential nephroprotective agent: Dose-related effects in renal ischemia-reperfusion injury

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DOI: https://doi.org/10.17305/bb.2025.13056

ABSTRACT

Ischemia-reperfusion injury (IRI) presents a complex pathophysiology characterized by oxidative stress and inflammation. Arbutin, widely recognized for its use in skin whitening, also exhibits antioxidant, anti-inflammatory, and anticancer properties. This study aimed to assess the potential protective effects of arbutin at two different doses against IRI in the kidneys. Twenty-four male Wistar albino rats were randomly assigned to four equal groups: Control, IRI, 250 mg/kg arbutin + IRI (AR250+IRI), and 1000 mg/kg arbutin + IRI (AR1000+IRI). Arbutin was administered orally via gavage for 14 days to ensure sub-acute application. Following left kidney nephrectomy, ischemia was induced in the right kidney using a non-traumatic clamp for 45 minutes, succeeded by 60 minutes of reperfusion. Blood and tissue samples were subsequently collected for analysis. In the IRI group, levels of malondialdehyde, myeloperoxidase, interleukin-1 beta, and creatinine were significantly elevated; these levels decreased in the groups receiving arbutin supplementation. Notably, ischemia-modified albumin, urea, superoxide dismutase (inhibition ratio), and tumor necrosis factor alpha levels were reduced in the AR1000+IRI group. Additionally, decreased levels of catalase and glutathione peroxidase were observed in the AR1000+IRI group. Histopathological examination revealed flattening, necrosis, degeneration, dilation, glomerular necrosis, sclerosis, Bowman capsule dilation, and interstitial hemorrhage in the IRI group. The AR250+IRI group exhibited mild cortical-medullary congestion and a slight increase in glomerular size. Conversely, the AR1000+IRI group displayed a histological appearance resembling that of the control group. In conclusion, arbutin demonstrates potential protective effects against IRI. Its use may be recommended prophylactically for individuals at risk of developing IRI.

Keywords: Ischemia-reperfusion injury, arbutin, malondialdehyde, myeloperoxidase, glutathione peroxidase, glomerular necrosis, bowman's capsule dilation, interstitial hemorrhage.

INTRODUCTION

Ischemia-reperfusion injury (IRI) is a dangerous health disorder that contributes to AKI (acute kidney injury) and leads to rapid renal failure and raises death rates. IRI, which is frequently observed in kidney transplantation, trauma, shock, and cardiovascular and urologic surgery, has no known therapeutic treatment [1]. IRI is related with high mortality and morbidity due to the sudden and transient obstruction of renal blood flow and currently has no proven treatment [2]. Much recent research has focused on the search for treatment or prevention strategies due to the challenging pathologic processes that characterize IRI and the lack of an effective drug [3-7].

AKI involves a variety of molecular and pathophysiological processes, such as inflammation, oxidative stress, fibrosis, apoptosis, and altered gene expression that trigger various signaling pathways. The cascade of inflammatory process, including the inflammatory events, complement activation, and activation of innate immunity, is an important part of IRI. IRI also induces necrosis and apoptosis, oxidative damage to DNA and proteins, and peroxidation of membrane lipids [8]. In addition, there is an increase in the expression of proteins associated with specific pathways that are directly linked to ferroptosis and oxidative stress in IRI. Renal fibrosis occurs as a result of ferroptosis, and oxidative stress caused by IRI [9]. Moreover, renal fibrosis and inflammation from AKI might lead to chronic kidney disease. Therefore, various therapeutic approaches have concentrated on reducing oxidative stress, inflammation, and fibrosis [10].

Arbutin, a hydroquinone glycoside determined in about 50 plant families, is one of the most widely used natural skin-lightening agents. Moreover, to this property, arbutin has some therapeutically important biological properties such as antioxidant, anti-inflammatory, antimicrobial, and anticancer potential [11]. Arbutin has inhibitory effects against oxidative stress-induced cytotoxicity that causes extensive tissue damage. A dose-dependent decrease in the production of reactive nitrogen and oxygen species is observed in cells treated with arbutin [12]. Arbutin exhibits good antioxidant properties as well as anti-inflammatory activity. Arbutin-containing microspheres exhibit antiinflammatory impacts by inhibiting Nuclear Factor Kappa B (NF-κB) signaling and antioxidative stress impacts by activating nuclear factor erythroid 2-related factor 2 (Nrf2) pathway [13].

IRI, the most common cause of AKI, has a complex pathophysiology based on inflammation and oxidative stress, which renders treatment ineffective. Arbutin, which has antioxidant activity and anti-inflammatory properties, is a natural skin whitening agent, but its therapeutic and preventive effects on a wide range of health problems are still unknown. This study purposed to investigate the protective role of arbutin, known for its anti-inflammatory and antioxidant properties, against IRI, a condition with a complex pathophysiology driven by oxidative stress and inflammation.

MATERIALS AND METHODS

Animals

The ethics committee decision required for the research was obtained from Aydın Adnan Menderes University (ADU) Animal Experiments Local Ethics Committee (ADUHADYEK). ADUHADYEK is an organization that supervises the conduct of experimental studies in accordance with ethical rules, and the approval and supervision of the study was carried out by ADUHADYEK. The experimental study was carried out by taking 24 male Wistar albino rats weighing 250-400 grams from ADU Faculty of Medicine Experimental Animal Production Center. Rats were divided into groups using a simple random number table. These rats were randomly divided into 4 groups as control group, IRI group, 250 mg/kg arbutin+IRI (AR250+IRI) and 1000 mg/kg arbutin+IRI (AR1000+IRI) groups with equal number of rats in each group.

Ischemia-reperfusion injury formation and application of arbutin supplementation

In the control group, IRI was not applied to the right kidney. IRI was induced in rats in the other three groups by clamping the right kidney. Arbutin (≥98% purity, Sigma-Aldrich, St. Louis, MO, USA; product number Y0000806, European Pharmacopoeia Reference Standard) was freshly dissolved in distilled water immediately prior to administration. Before IRI, rats in the AR250+IRI group received 250 mg/kg arbutin orally by gavage for 14 days (sub-acute application), and those in the AR1000+IRI group received 1000 mg/kg arbutin. The gavage volume was adjusted to 1 mL/100 g body weight to ensure accurate dosing across animals.

For the experimentally created renal IRI model, rats were anesthetized with intraperitoneal ketamine (90 mg/kg; 60 mg/mL) and xylazine (10 mg/kg; 20 mg/mL). After anesthesia, the abdomens were opened, and the left kidney was removed and ligated to prevent bleeding, leaving only the right kidney. A non-traumatic clamp was then placed on the right renal artery, creating 45 minutes of ischemia followed by a 60-minute reperfusion period (Figure 1). The 45-minute ischemia period was chosen because renal histological damage and functional loss can be reliably observed after this period. For example, based on the findings of a study by Kapisiz et al., they observed that damage began within 4 hours and peaked 24 hours after 45 minutes of ischemia [14]. In many studies on renal IRI in the literature, 45 minutes of ischemia and 60 minutes of reperfusion are among the preferred protocols [15-18]. At the end of reperfusion, intracardiac blood samples were collected, and kidney tissue samples were stored at -80°C until biochemical analyses.

Biochemical analyzes

During biochemical analyses, the laboratory team was not informed which group the samples belonged to (blinding was performed). Blood samples were centrifuged at 3000 rpm for 10 minutes. Creatinine and urea analyses were performed from the serum samples obtained because of centrifugation. For biochemical analyses, tissue samples thawed at room temperature were homogenized in phosphate buffer (50 mM, pH 7.0) at a ratio of 1:10 (w/v). Tissue homogenates were centrifuged at 4 °C for 15 minutes. Protein concentrations in the supernatants were determined using the Bradford method, and all ELISA results were normalized to protein content. ELISA assays were performed in duplicate in accordance with the manufacturers' protocols, and mean values were used for statistical analyses.

Supernatants obtained after centrifugation were analyzed for malondialdehyde (K739-100, Biovision, USA) [19], superoxide dismutase (SOD) inhibition rate (K335-100, Biovision, USA) [20], myeloperoxidase (K744-100, Biovision, USA) [21], Glutathione peroxidase (GPx) (K762-100, Biovision, USA) [22], tumor necrosis factor alpha (TNF-α) (K1052-100, Biovision, USA) [23], interleukin-1 Beta (IL-1β) (K4796-100, Biovision, USA) [24] and ischemia modified albumin (IMA) (ER1108, FineTest, China) [25]. The protocols of the kits used were applied and readings were made on the Enzyme-Linked Immuno Sorbent Assay device.

Histopathological analyses

For histopathological analyses, tissue samples were fixed in 10% neutral formaldehyde at 24 °C. The identified tissues were embedded in paraffin blocks. Paraffin blocks were cut at 5-micron thickness using a microtome. Hematoxylin and eosin staining was performed. The sections were examined by normal light microscopy. Routine staining method was used for staining specimens. For hematoxylin and eosin staining, the samples were kept in xylene pools for 5 minutes followed by 4 minutes for a total of 9 minutes. For washing, the samples were kept in 100% and 80% alcohol solutions for 2 minutes and then washed with distilled water. To prepare for microscopic analysis, all samples were immersed in hematoxylin stain for 3 minutes and then washed and immersed in eosin stain for 1 minute.

Ethical statement

The ethics committee decision required for the study was obtained from ADUHADYEK (Decision number: 64583101/2023/05). This study was conducted and reported in accordance with ARRIVE guidelines to ensure transparent and complete reporting of animal experiments [26].

Statistical analysis

IBM SPSS Statistics 22.0 was used to conduct statistical analyses (IBM Corp., Armonk, NY, USA). Biochemical variables were evaluated using the Shapiro-Wilk test for normal distribution and the Levene test for homogeneity of variances. Data were reported as mean±standard deviation. Intergroup differences in the variables were analyzed using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. Additionally, Pearson correlation analysis was performed. Statistical procedures were conducted in accordance with standard biostatistical practices [27].

RESULTS

Effect of ischemia-reperfusion injury on biochemical parameters

Malondialdehyde, myeloperoxidase, IMA, TNF-α, IL-1β, creatinine, urea and SOD (inhibition rate) averages were highest in the IRI group, while the lowest averages were found in the control group. Malondialdehyde, myeloperoxidase, IMA, TNF-α, IL-1β, creatinine, urea and SOD (inhibition rate) averages were higher in the IRI

group compared to the control group (p=0.001, p<0.001, p=0.015, p=0.006, p<0.001, p<0.001, p<0.001, respectively) (Table 1, Figures 2 and 3). The lowest GPx and catalase enzyme activity values were found in the IRI group, while the highest mean was found in the control group. Catalase and GPx enzyme activity values were lower in the IRI group compared to the control group (p=0.001, p<0.001, respectively) (Table 1 and Figure 2).

Effect of different doses of arbutin supplementation on ischemia-reperfusion injury

Malondialdehyde, myeloperoxidase, IL-1β and creatinine averages were lower in the AR250+IRI group compared to the IRI group (p=0.028, p=0.001, p=0.020, p=0.042, respectively) (Table 1, Figures 2 and 3). Catalase enzyme activity values were higher in the AR250+IRI group compared to the IRI group (p=0.049) (Table 1 and Figure 2).

Malondialdehyde, myeloperoxidase, IMA, TNF-α, IL-1β, creatinine, urea and SOD (inhibition rate) averages were lower in the AR1000+IRI group compared to the IRI group (p=0.001, p<0.001, p=0.043, p=0.021, p=0.043, p=0.004, p=0.005, p=0.007, respectively) (Table 1, Figures 2 and 3). Catalase and GPx enzyme activity levels were higher in the AR1000+IRI group compared to the IRI group (p=0.004, p=0,027, respectively) (Table 1 and Figure 2).

Comparison of AR250+IRI and AR1000+IRI groups with each other and with the control group

Creatinine and urea averages were higher in the AR1000+IRI group compared to the control group (p=0.025, p<0.001, respectively) (Table 1 and Figure 3).

Myeloperoxidase, creatinine and urea were higher in the AR250+IRI group compared to the control group (p=0.044, p=0.002, p<0.001, respectively) (Table 1, Figures 2 and 3). Catalase and GPx enzyme activity levels were lower in the AR250+IRI group compared to the control group (p=0.004, p=0.039, respectively) (Table 1 and Figure 2).

Correlation of biochemical findings with group scoring

The groups were scored as follows: 1 = IRI group, 2 = Low-dose Arbutin+IRI (AR250+IRI) group, 3 = High-dose Arbutin+IRI (AR1000+IRI) group, and 4 = Healthy control group. Correlation analysis was performed between biochemical

findings and these group scoring. Analysis results are presented in Table 2. A negative correlation was found between group score and malondialdehyde, myeloperoxidase, IMA, TNF-α, IL-1β, SOD (inhibition rate), creatinine and urea levels, and a positive correlation was found between catalase and GPx levels (Table 2).

Histopathological findings

Analyses using hematoxylin and eosin staining and light microscopy (H&E, x200) revealed no histopathologic findings in the control group (Figure 4A). Flattening, necrosis, degeneration, dilatation, glomerular necrosis and sclerosis, Bowman's capsule dilatation, interstitial hemorrhage were observed in the IRI group (H&E, x200) (Figure 4B). In the AR250+IRI group, mild cortical-medullary congestion and a small enlargement of glomerular size were observed (H&E, x200) (Figure 4C). A similar appearance was observed in the AR1000+IRI group compared to the control group (H&E, x200) (Figure 4D).

DISCUSSION

IRI is a complex pathological process affected by many factors such as metabolic stress, oxidative stress, inflammatory immune responses, leukocyte infiltration, and programmed cell death. IRI is not only a consequence of these factors but also a trigger of various biological responses such as oxidative stress, inflammation, and programmed cell death [28]. Among these biological responses, oxidative stress leads to the accumulation of reactive oxygen species (ROS) and disruption of intracellular redox balance. This disrupts the integrity of the cell membrane and triggers lipid peroxidation. Increased lipid peroxidation causes structural changes in cell membranes and mitochondrial disruption, leading to oxidative cell death [29]. Malondialdehyde, an important biomarker of lipid peroxidation, increases significantly during IRI and this increase is closely associated with secondary organ damage [30].

In this research, malondialdehyde values were increased in the kidney tissue of rats injured with IRI and decreased in rats previously supplemented with different doses of arbutin.

IMA, a marker for acute ischemia approved by the US Food and Drug Administration, plays a role in the detection of acute ischemia before necrosis [31]. Moreover, IMA

levels increase in parallel with the ischemia duration in IRI, and this increase is supported by histopathological damage results [32]. Another important parameter whose levels increase as a result of inflammation and oxidative stress associated with IRI is myeloperoxidase, which plays a role in the antimicrobial activity of neutrophils and is a significant component of the immune response in various inflammatory processes, including phagocytosis [33]. Myeloperoxidase reacts with the SOD substrate, superoxide, and uses superoxide as a cofactor to continue producing hypochlorous acid. SOD, in turn, uses superoxide as a substrate to produce less ROS [34]. Overproduction of superoxide, used as a substrate by SOD and as a cofactor by myeloperoxidase, is the source of increased damage in IRI [35].

In this study, myeloperoxidase levels increased in the kidney tissue of animals injured with IRI and decreased in animals previously supplemented with different doses of arbutin. Moreover, IMA and SOD (inhibition rate) levels increased in the kidney tissue of rats injured with IRI and decreased in rats previously supplemented with 1000 mg/kg arbutin.

A recent study reported that arbutin, which has anti-inflammatory and antioxidant effects, alleviates acute liver injury (ALI) by inhibiting apoptosis and inflammation [36]. Another recent study reported that arbutin supplementation increased GPx and catalase levels and improved antioxidant activity [37]. Furthermore, endogenous catalase, important for cellular antioxidant defenses, is critical for preventing IRI [38]. In a study in which experimental testicular IRI was induced, oxidative stress parameters were reported to increase with IRI, while arbutin supplementation increased catalase levels and reduced oxidative stress parameters [39]. A study investigating the protective impact of arbutin on ALI reported that arbutin supplementation increased GPx and total antioxidant capacity levels and had hepatoprotective activity [40]. A study investigating the neuroprotective impact of arbutin against oxidative stress and neuroinflammation reported that arbutin supplementation increased antioxidant status (e.g. glutathione, catalase, and SOD) and could protect against stroke and ischemic injuries [41].

In this research investigating the potential nephroprotective properties of arbutin, catalase values were decreased in the kidney tissue of rats injured with IRI and increased in rats previously receiving different doses of arbutin supplementation.

Moreover, GPx values decreased in the kidney tissue of rats injured with IRI and increased in rats previously supplemented with 1000 mg/kg arbutin.

The first step of IRI is mediated by ischemia, which promotes the production of ROS in cells. This further promotes the activation of pro-inflammatory signaling cascades, including the production of damage-related molecular patterns such as TNF-α, interferon, interleukin-6, inducible nitric oxide synthase, TLR9/NF-κB pathway, etc [42]. Morphological changes, apoptosis, and inflammation occur in kidneys with IRI. In addition, the values of proinflammatory cytokines such as IL-1β and TNF-α are significantly increased by IRI [43]. Increased values of these proinflammatory cytokines have been reported in many studies in IRI, the most common cause of AKI [44-46].

In this study, IL-1β levels increased in the kidney tissue of animals injured with IRI and decreased in rats previously supplemented with different doses of arbutin. Moreover, TNF-α levels increased in the kidney tissue of animals injured with IRI and decreased in rats previously supplemented with 1000 mg/kg arbutin.

The most widely accepted parameters for the assessment of renal failure are creatinine, urea and glomerular filtration rate [47]. Increases in values of creatinine and urea, among these markers, can be evaluated to indicate increased susceptibility to tubular damage and tissue damage in IRI-induced acute kidney injury [48]. Furthermore, serum urea and creatinine levels are biochemical parameters frequently used to assess renal function in experimental animal models in which IRI is induced [49].

In this research, creatinine levels increased in the blood serum of rats injured with IRI and decreased in rats previously supplemented with different doses of arbutin. Moreover, urea levels increased in the blood serum of animals injured with IRI and decreased in rats previously supplemented with 1000 mg/kg arbutin.

In this study, an inverse relationship was found between group scoring and malondialdehyde, myeloperoxidase, IMA, TNF-α, IL-1β, SOD (inhibition rate), creatinine and urea levels, and a positive relationship was found between catalase and GPx levels

Tubular cell death associated with apoptosis and necrosis is one of the main pathological features of renal IRI [50]. AKI is usually characterized by simultaneous

rupture of plasma membranes of cells in specific segments of the nephron, also called “acute tubular necrosis” [51]. In addition, histopathological findings of renal IRI include erythrocyte congestion and widespread cell swelling in both the medulla and cortex, marked cell apoptosis/necrosis in the medulla, and cast formation [52].

In this study, flattening, necrosis, degeneration, dilatation, glomerular necrosis and sclerosis, Bowman's capsule dilatation and interstitial hemorrhage were observed in the kidney tissue of animals injured by IRI, while no histopathological findings were observed in the kidney tissue of animals prophylactically treated with 1000 mg/kg arbutin. In addition, mild cortical-medullary congestion and a small enlargement of glomerular size were observed in the kidney tissue of rats prophylactically treated with 250 mg/kg arbutin.

This study has several limitations that must be acknowledged. First, the histological assessment was qualitative, and a blinded lesion scoring system could not be applied. This limits the quantitative interpretation of tissue damage. Second, only two widely spaced arbutin doses were tested, limiting the establishment of a definitive dose-response relationship. Finally, the low sample size due to the study's preclinical experimental animal model may limit the generalizability of the findings. However, this study also has strengths. The results can be used as a reference source for future human clinical studies, quantitative histological assessments, or experimental studies investigating different dose ranges. Therefore, this study makes an important contribution to guiding future research.

CONCLUSION

Increased oxidative stress, impaired renal function tests, histopathological damage and signs of inflammation associated with IRI exposure were attenuated by prophylactic arbutin supplementation. In addition, IRI-induced decreased antioxidant enzyme activities were found to increase with arbutin supplementation. These data suggest that arbutin may have potential protective effects against IRI. Therefore, arbutin can be considered as a potential supportive agent for prophylactic purposes.

Conflicts of interest: Authors declare no conflicts of interest.

Funding: Authors received no specific funding for this work.

Data availability: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Submitted: July 30, 2025

Accepted: August 30, 2025

Published online: September 18, 2025

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TABLES AND FIGURES WITH LEGENDS

Table 1. Biochemical parameters of the study groups

Parameters*	Control	IRI	AR250+IRI	AR1000+IRI
MDA (nmol/g)	286.83±40.24 [a]	428.00±55.71 [b]	335.50±54.39 [a]	294.17±56.06 [a]
MPO (U/g)	375.83±46.63 [a]	631.33±62.29 [b]	474.00±83.34 [c]	422.33±33.11 [a,c]
IMA (ng/g)	14.83±4.79 [a]	21.83±3.87 [b]	19.33±3.14 [a,b]	15.83±2.14 [a]
SOD inhibition (%)	14.50±5.09 [a]	32.17±6.31 [b]	22.83±7.36 [a,b]	19.67±4.23 [a]
CAT (U/g)	445.50±76.61 [a]	220.50±68.04 [b]	330.67±78.65 [c]	375.83±43.53 [a,c]
GPx (U/g)	799.17±92.38 [a]	523.00±124.72 [b]	561.17±92.88 [b,c]	708.83±103.68 [a,c]
TNF-α (pg/g)	559.50±78.53 [a]	766.17±131.67 [b]	654.67±61.86 [a,b]	586.00±93.17 [a]
IL-1β (pg/g)	438.33±126.18 [a]	733.83±96.07 [b]	539.50±53.07 [a]	561.83±123.37 [a]
Creatinine (mg/dL)	0.46±0.09 [a]	0.95±0.15 [b]	0.76±0.13 [c]	0.68±0.09 [c]
Urea (mg/dL)	13.67±3.76 [a]	34.33±6.25 [b]	27.83±3.06 [b,c]	25.00±2.68 [c]

Different superscript letters ([a], [b], [c]) in the same row indicate statistically significant differences between groups ($p<0.05$). Intergroup differences were analyzed by one-way ANOVA with Tukey's post-hoc test. *Creatinine and urea results were obtained from blood serum, while other parameters were obtained from tissue homogenate and normalized according to tissue quantity. Abbreviations: IRI: Ischemia-reperfusion injury; AR250: 250 mg/kg Arbutin; AR1000: 1000 mg/kg Arbutin; MDA:

Malondialdehyde; MPO: Myeloperoxidase; IMA: Ischemia modified albumin; SOD: Superoxide dismutase (Inhibition rate); CAT: Catalase; GPx: Glutathione peroxidase; TNF-α: Tumor necrosis factor alpha; IL-1β: Interleukin-1 Beta.

Table 2. Correlation analysis results of group scores and biochemical findings

Parameters	**Group score***	
	Correlation coefficient	***p***
Malondialdehyde	-0.706	<0.001
Myeloperoxidase	-0.828	<0.001
Ischemia modified albumin	-0.633	0.001
Superoxide dismutase (Inhibition rate)	-0.750	<0.001
Catalase	0.745	<0.001
Glutathione peroxidase	0.784	<0.001
Tumor necrosis factor alpha	-0.653	0.001
Interleukin-1 Beta	-0.678	<0.001
Creatinine	-0.837	<0.001
Urea	-0.866	<0.001

*The groups were scored as follows: 1 = IRI group, 2 = Low-dose Arbutin+IRI (AR250+IRI) group, 3 = High-dose Arbutin+IRI (AR1000+IRI) group, and 4 = Healthy control group. Abbreviation: IRI: Ischemia-reperfusion injury.

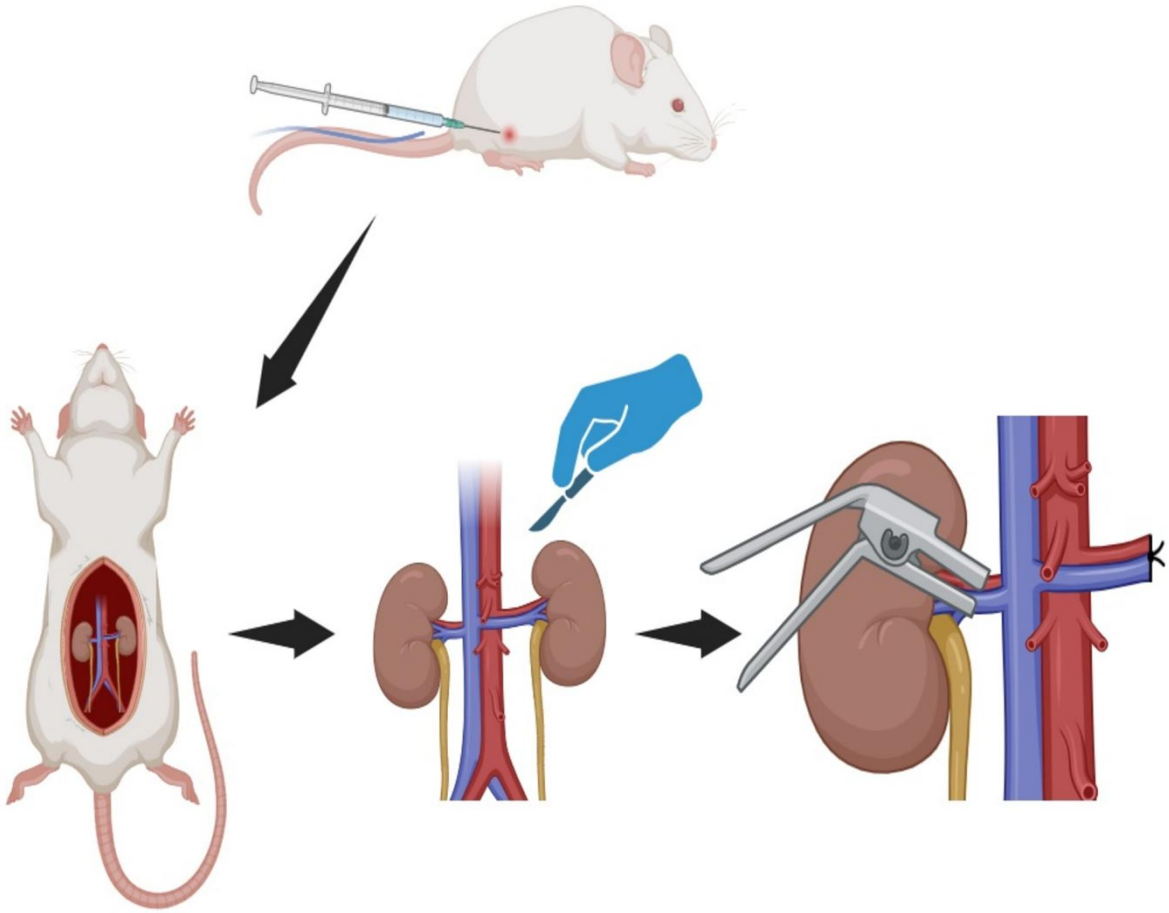

Figure 1. Schematic view of the unilateral renal ischemia-reperfusion (IRI) model in rats. After left kidney nephrectomy, ischemia was induced in the right kidney with a non-traumatic clamp for 45 minutes, followed by 60 minutes of reperfusion.

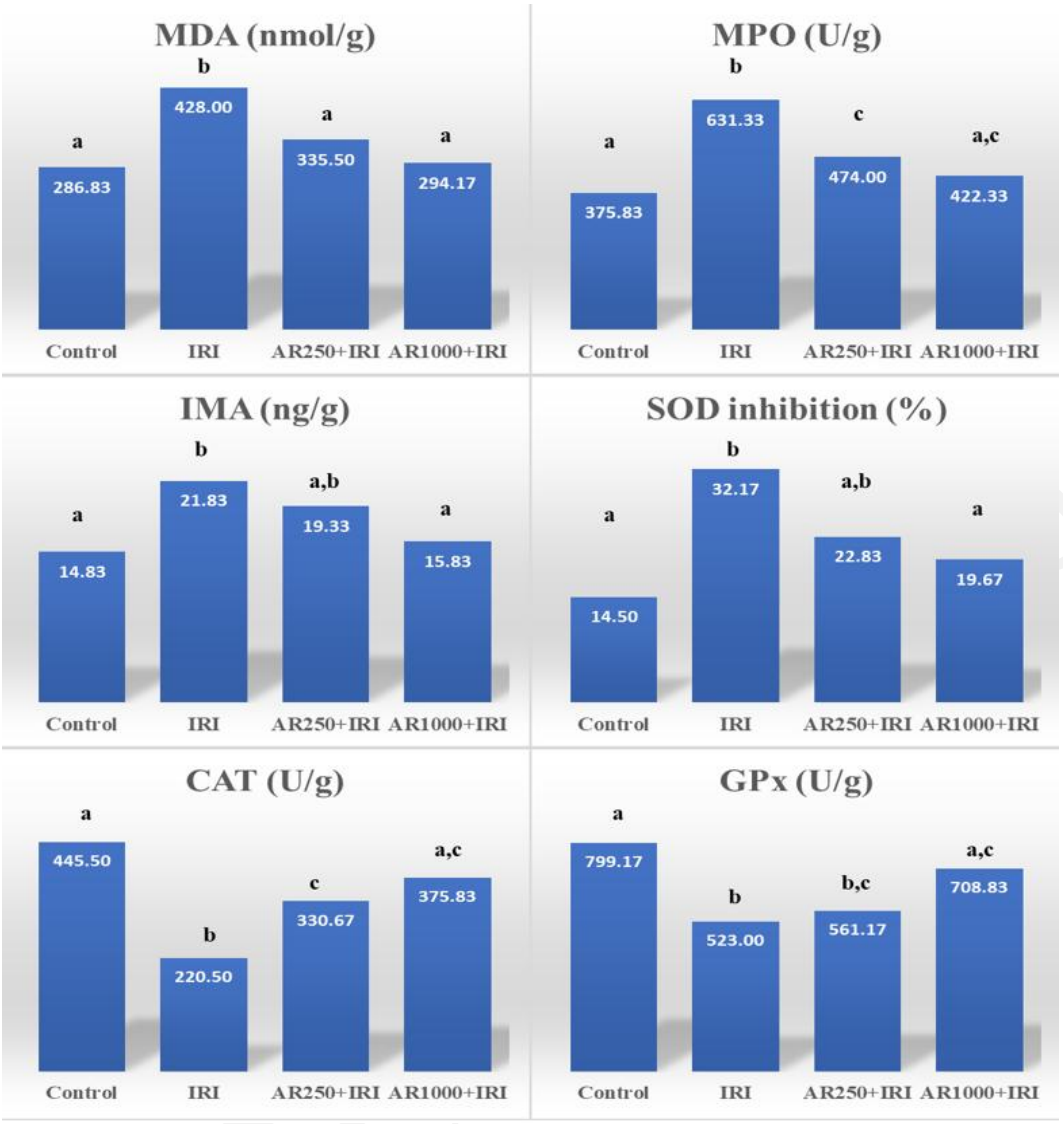

Figure 2. Bar graphs of biochemical results of groups-1. Different superscript letters ([a], [b], [c]) in the same row indicate statistically significant differences between groups (p<0.05). Intergroup differences were analyzed by one-way ANOVA with Tukey's post-hoc test. *Creatinine and urea results were obtained from blood serum, while other parameters were obtained from tissue homogenate and normalized according to tissue quantity. Abbreviations: MDA: Malondialdehyde; MPO: Myeloperoxidase; IMA: Ischemia modified albumin; SOD: Superoxide dismutase (Inhibition rate); CAT: Catalase; GPx: Glutathione peroxidase.

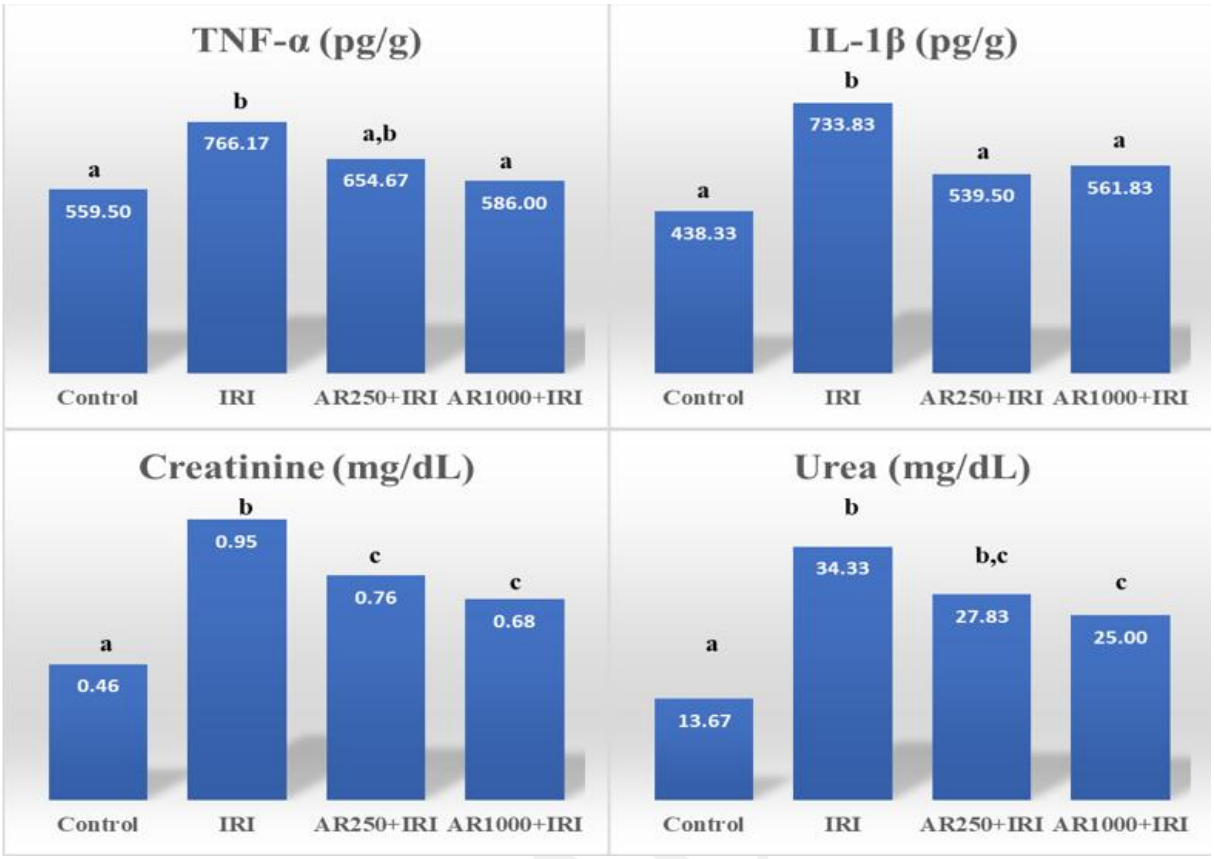

Figure 3. Bar graphs of biochemical results of groups-2. Different superscript letters ([a], [b], [c]) in the same row indicate statistically significant differences between groups (p<0.05). Intergroup differences were analyzed by one-way ANOVA with Tukey's post-hoc test. * Creatinine and urea results were obtained from blood serum, while other parameters were obtained from tissue homogenate and normalized according to tissue quantity. Abbreviations: TNF-α: Tumor necrosis factor alpha; IL-1β: Interleukin-1 Beta.

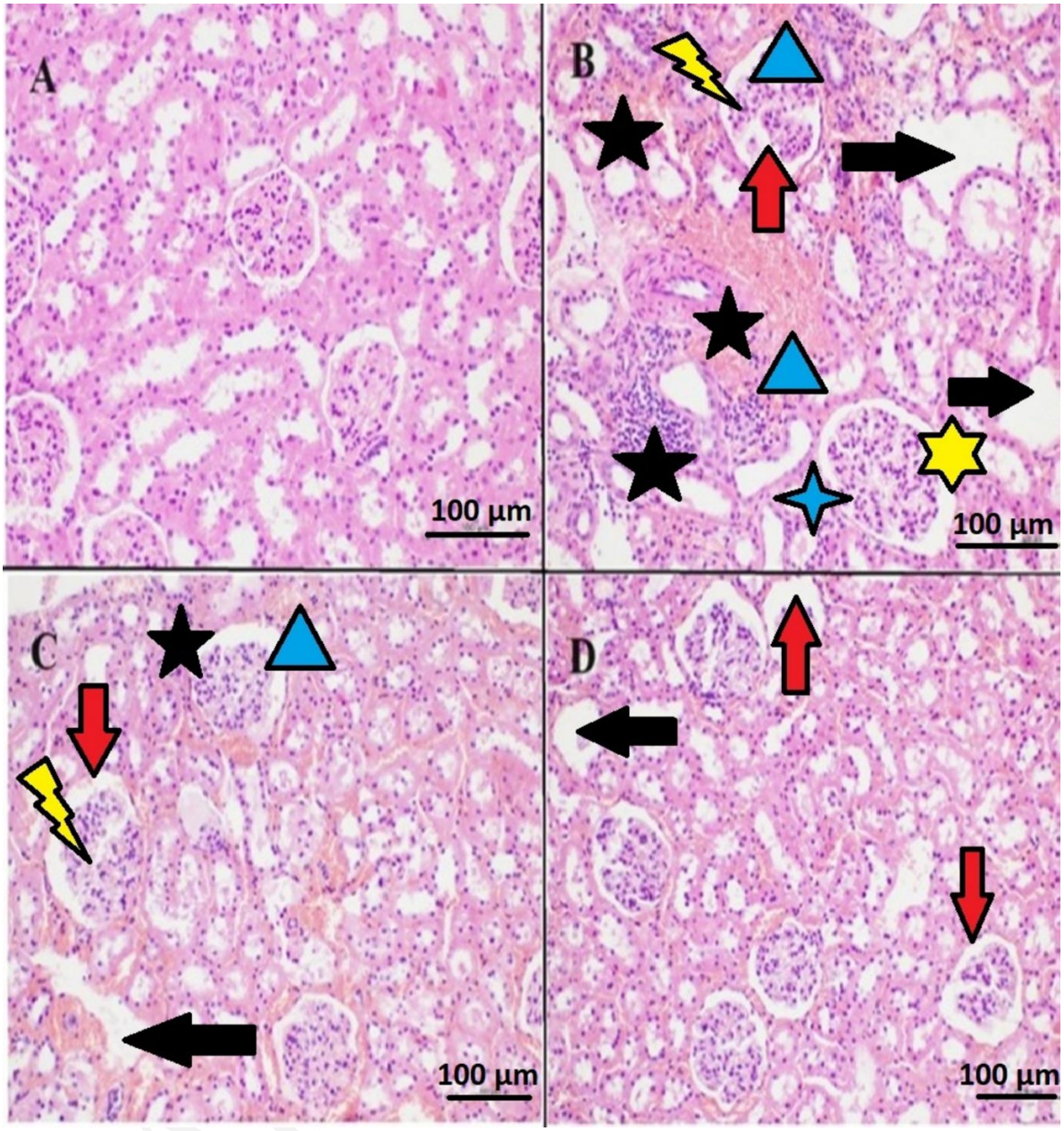

Figure 4. Histopathological kidney tissue images. (A) Control group; (B) Ischemia-reperfusion injury (IRI) group; (C) 250 mg/kg arbutin+IRI (AR250+IRI) group; (D) 1000 mg/kg arbutin+IRI (AR1000+IRI) group. Horizontal black arrow: Dilation and necrosis; Vertical red arrow: Bowman's capsule dilatation, flattening, and necrosis; Blue arrowhead-triangle: Degeneration and hemorrhage; 4-sided blue star: Sclerosis; 5-sided black star: Cellular infiltration, hemorrhage, and necrosis; 6-sided red star: Cortical-medullary congestion; Lightning-shaped yellow arrow: Enlargement in glomerular size. *Sections were taken at 2-3 µm thickness and stained with hematoxylin and eosin. At least 10 high-power fields were examined for each kidney section. Photomicrographs of all kidney tissue samples were taken at x200 magnification in H&E-stained preparations.