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Evaluation of the Pathophysiological Effects of Vitamin C and Broccoli Extract Pretreatment on Hepatic and Renal Ischemia–Reperfusion Injury in Wistar Rats

Danial Fatehi[1], Mahdieh Raeeszadeh[2*], Peyman Mohammadzadeh[3]

1. *Graduate of Faculty of Veterinary Sciences (D.V.M), Sa.C., Islamic Azad University, Sanandaj, Iran*
2. *Department of Basic Sciences, Sa.C., Islamic Azad University, Sanandaj, Iran*
3. *Department of Pathobiology, Sa.C., Islamic Azad University, Sanandaj, Iran*

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***Corresponding author:**
Mahdieh Raeeszadeh,
Department of Basic Sciences, Sa.C., Islamic Azad University, Sanandaj, Iran

Email: mraeeszadeh@iau.ac.ir

ABSTRACT

Background & Objective: Antioxidants play a crucial role in mitigating ischemia-reperfusion (I/R) induced tissue injury. Comparing the therapeutic efficacy of natural and synthetic antioxidants holds substantial clinical importance. This study aimed to evaluate and compare the protective effects of broccoli extract and vitamin C against hepatic and renal I/R injury in Wistar rats.

Materials & Methods: Bioactive compounds in broccoli extract were identified by gas chromatography mass spectrometry (GC–MS). Hepatic and renal I/R models were established in rats and divided into four groups per organ (n = 8): control, I/R-only, I/R with broccoli extract pretreatment, and I/R with vitamin C pretreatment. Serum liver enzymes (AST, ALT, ALP) and renal function markers (creatinine, urea) were measured. Tissue oxidative stress markers (malondialdehyde, MDA), antioxidant enzyme activities (catalase and superoxide dismutase), and pro-inflammatory cytokines (IL-6 and TNF-α) were assessed. Histopathological analyses were performed on stained tissue sections.

Results: GC–MS analysis identified thymol and sulforaphane as the major antioxidant components of broccoli extract. I/R injury significantly increased serum AST and ALT levels in the liver and elevated creatinine and urea levels in the kidney. Pretreatment with broccoli extract or vitamin C significantly attenuated these changes. Treated groups showed increased antioxidant enzyme activities and reduced MDA levels compared with I/R-only groups. IL-6 and TNF-α expression was more pronounced in renal than hepatic tissues. Histopathological findings confirmed reduced tissue damage and inflammation following antioxidant pretreatment.

Conclusion: Broccoli extract provided greater protection than vitamin C, particularly against hepatic I/R injury, likely due to its combined antioxidant and anti-inflammatory effects, supporting its potential as a natural therapeutic agent.

Keywords: Ischemia/reperfusion (I/R), Liver, Kidney, Broccoli, Vitamin C, Down-regulatio

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1. Introduction

Hepatic and renal ischemia/reperfusion (I/R) injury is a major cause of transplant failure, occurring in nearly 50% of transplantation procedures (1). Interruptions in blood flow during surgery, resection, or trauma result in hypoxia and the accumulation of metabolic waste, leading to ischemic tissue damage (2). Subsequent restoration of circulation activates lysosomal proteases, which can trigger apoptosis (3). The delicate balance between cellular survival and death ultimately determines postoperative organ function (4).

The pathogenesis of I/R injury proceeds through two distinct phases: an initial neutrophil-independent phase characterized by excessive free radical generation, followed by a neutrophil-mediated inflammatory phase. During reperfusion, the production of reactive oxygen species (ROS) increases markedly, and these species interact with nucleic acids, proteins, and membrane lipids, impairing their structural and functional integrity (5). Neutrophil recruitment through chemokine signaling further amplifies injury via the release of ROS and pro-inflammatory cytokines such as TNF-α, which exert direct cytotoxic effects (6). The overproduction of ROS disrupts endogenous antioxidant defense systems, highlighting the critical role of exogenous antioxidants in restoring redox homeostasis and limiting tissue injury (7).

Vitamin C, a potent water-soluble antioxidant, mitigates oxidative stress by donating electrons to neutralize free radicals (9). Broccoli (*Brassica oleracea* var. *italica*) contains a diverse range of antioxidant compounds, including vitamins C and E, flavonoids, carotenoids, and glucosinolates, contributing to its substantial free radical–scavenging capacity (8). The phenolic and flavonoid constituents of broccoli have been shown to effectively neutralize oxidative agents, positioning it as a promising candidate for combating oxidative damage.

Despite the established antioxidant properties of both agents, direct comparative studies examining the effects of broccoli extract and vitamin C in hepatic and renal I/R models remain limited. Therefore, this study aimed to evaluate and compare their protective efficacy using biochemical, molecular, and histopathological analyses.

2. Materials and Methods

2.1 Preparation of Broccoli Extract

Three 100 gr batches of broccoli powder were each mixed with 1 L of 80% methanol and agitated for 48 hours. The mixtures were then filtered, and the filtrates were concentrated using a rotary evaporator (Model STRIKE285, Italy) to obtain a dry powder extract. The extract was administered at a dose of 300 mg/kg (9).

2.2 GC–MS Analysis

Fifty gr of broccoli powder were extracted with 50 mL of 99% methanol for 4 hours, left to stand for 24 hours, and subsequently filtered. A 10 mL aliquot of the filtrate was injected into an Agilent 7890B gas chromatograph coupled with a 5977A mass selective detector (MSD). Separation was performed on an HP-5 ms column (30 m × 0.25 mm, 0.25 µm). The injector temperature was set at 280 °C, and the oven temperature was programmed from 50 °C to 260 °C at a rate of 10 °C/min. Compound identification was carried out using Kovats retention indices and comparison with NIST library mass spectra (10, 11).

2.3 Study Design and Animal Grouping

A total of sixty-four male Wistar rats (200–230 gr) were randomly divided into eight groups (n = 8 per group) following a one-week acclimation period. Animals had ad libitum access to standard feed and water, and were maintained under controlled conditions (12 h light/dark cycle, 22 ± 3 °C). All experimental procedures were approved by the Ethics Committee of Islamic Azad University of Sanandaj (IR.IAU.SDJ.REC.1400.074) (12, 13).

2.3.1 Liver Groups include

Control (C), I/R-L: Ischemia/reperfusion without treatment, I/R-LB: I/R with broccoli extract pretreatment (300 mg/kg) and I/R-LC: I/R with vitamin C pretreatment (300 mg/kg).

2.3.2 Kidney Groups

Control (C), I/R-K: Ischemia/reperfusion without treatment, I/R-KB: I/R with broccoli extract pretreatment (300 mg/kg) and I/R-KC: I/R with vitamin C pretreatment (300 mg/kg).

Vitamin C solution (500 mg/5 mL; Aburaihan Pharmaceutical Company) was freshly prepared and administered daily by gavage for 21 days in the groups at fixed times by a blinded researcher. Histological evaluations were performed by two independent, blinded pathologists.

2.4. Induction of Ischemia/Reperfusion

2.4.1. Liver Model

Rats were anesthetized using ketamine (100 mg/kg) and xylazine (10 mg/kg). Partial hepatic ischemia was induced by clamping the portal triad for 45 minutes, followed by 90 minutes of reperfusion (14, 15).

2.4.2. Kidney Model

Renal ischemia was induced by clamping both renal pedicles for 45 minutes under anesthesia, followed by 90 minutes of reperfusion (16).

2.5. Biochemical and Oxidative Stress Assessments

Serum levels of AST, ALT, ALP, creatinine, and urea were determined using commercial diagnostic kits (17-21). Tissue homogenates were analyzed for malondialdehyde (MDA) concentration and antioxidant enzyme activities, including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx). Gene expression of IL-6 and TNF-α was quantified by quantitative real-time PCR (qRT-PCR) using the validated following primers (22–26).

Gene	Forward Primer (5′–3′)	Reverse Primer (5′–3′)	Accession No. (Amplicon Size)
IL-6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC	NM_031168 (76 bp)
TNF-α	CCTCCCTCTCATCAGTTCTA	ACTTGGTGGTTTGCTACGAC	NM_013693.1 (102 bp)

2.6. Histopathological Examination

Tissue samples were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 4 µm thickness, and stained with hematoxylin and eosin (H&E). Sections were examined microscopically for evidence of hemorrhage, hyperemia, and inflammation (27).

2.7. Statistical Analysis

Data are expressed as mean ± standard deviation (SD). The Kolmogorov–Smirnov test was applied to assess normality. Parametric data were analyzed using one-way ANOVA followed by Duncan's post-hoc test, while non-parametric data were analyzed using the Kruskal–Wallis test followed by the Mann–Whitney U test. Statistical significance was set at $p < 0.05$, $p < 0.01$, and $p < 0.001$. All analyses were performed using SPSS software version 26.0.

3. Result

GC–MS analysis indicated that the broccoli extract predominantly contained phenol (15.92%) and 1-isothiocyanato-4-[methylsulfinyl] butane (20.89%), with other constituents present in lower proportions (Table 1).

Table 2 summarizes the changes in hepatic enzyme levels. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were highest in the I/R-L group and lowest in the control (C) group ($p = 0.001$). In contrast, alkaline phosphatase (ALP) levels did not differ significantly among the groups. Representative histological sections of liver tissue are shown in Figure 1, illustrating pathological features such as portal vein hyperemia, hemorrhage, and inflammatory cell infiltration.

With respect to renal function, serum creatinine concentrations were highest in the I/R-K group and lowest in the control group, while urea levels followed a similar trend. Statistically significant differences were observed among most experimental groups ($p < 0.001$), except between I/R-KB and I/R-KC (Table 2). Histopathological examination of kidney sections revealed hyperemia and inflammatory cell infiltration, consistent with the biochemical findings (Figure 2).

Different superscript letters (a–d) within the same row indicate statistically significant differences among groups ($p < 0.05$); AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase.

In the Control group, normal tissue was preserved, with no lesions or hyperemia observed in the central vein. In contrast, I/R-L livers exhibited marked central vein dilation, hyperemia, extensive hemorrhage, and prominent inflammatory cell infiltration. Pretreatment with broccoli extract (I/R-LB) resulted in only mild inflammatory cell infiltration, whereas vitamin C pretreatment (I/R-LC) was associated with moderate venous congestion and inflammatory cell presence.

Kidneys in the I/R-K group exhibited pronounced inflammatory cell infiltration and hyperemia. In contrast, both the C and broccoli extract–pretreated (I/R-KB) groups preserved normal glomerular and tubular architecture, whereas vitamin C–pretreated (I/R-KC) kidneys displayed mild venous hyperemia. As shown in Figure 3A, liver lesion scores were highest in the I/R-L group and lowest in the C and I/R-LB groups. In kidney histology, hyperemia and inflammatory cell infiltration were key indicators, with the I/R-K group exhibiting significantly higher inflammatory cell counts than all other groups. Hemorrhage severity also differed significantly between the C and I/R-KB groups, as well as across the experimental groups. Kidney histopathological scores are summarized in Figure 3, demonstrating that the I/R-K kidneys sustained the most severe tissue damage.

Figure 4 illustrates the alterations in oxidative stress parameters and pro-inflammatory biomarkers. MDA concentrations were highest in the I/R-L group and lowest in the control (C) group. Catalase (CAT) activity in liver tissue was greatest in controls (1.652 ± 92.25 U/g) and lowest in I/R-L (30.25 ± 0.853 U/g). Superoxide dismutase (SOD) activity followed a similar trend, being highest in controls and lowest in I/R-L ($p < 0.001$). Glutathione peroxidase (GPx) activity reached its maximum in controls and minimum in the I/R-LC group. Interleukin-6 (IL-6) expression was lowest in controls (1.02 ± 0.170) and highest in I/R-L (3.30 ± 0.244), whereas the I/R-LB group exhibited lower IL-6 levels than both I/R-L and I/R-LC (Figure 4-E). Tumor necrosis factor-alpha (TNF-α) expression was the highest in I/R-LC (2.30 ± 0.108) and the lowest in controls (0.56 ± 0.129) (Figure 4-F).

Kidney oxidative stress markers and pro-inflammatory gene expression are presented in Figure 5. Malondialdehyde (MDA) levels were highest in the I/R-K group (7.40 ± 1.687) and lowest in the control (C) group (0.96 ± 0.052). CAT and SOD activities were maximal in controls and minimal in I/R-K ($p < 0.001$). GPx activity was greatest in controls and lowest in the I/R-KC group. Expression of pro-inflammatory genes was highest in I/R-K, lowest in controls, and intermediate in the I/R-KB group.

Table 1. Compounds from broccoli extract by the GC-MS technique.

Compound number (#)	RT (min)	Name	Percent	Retention Index
1	2.20	2-Heptenal	2.19	526
2	2.38	Dimethyl trisulfide	3.17	614
3	3.49	2,4-Dihydroxy-2,5-dimethyl -3 (2H)-furan-3-one	3.24	984
4	5.59	S-Methyl methanethiosulfinate	2.99	1063
5	8.02	4H-Pyran-4-one, 2,3-dihydro-3,5- dihydroxy-6-methyl	2.97	1151
6	9.35	2-Methoxy-4-vinylphenol	2.96	1314
7	10.87	DL-Proline, 5-oxo-, methyl ester	2.11	1385
8	11.17	2-Hydroxy-1-(1'-pyrrolidiyl)-1-buten-3-1	6.68	1515
9	12.58	Diphenylmethanone	2.02	1637
10	13.38	Pentadecafluorooctanoic acid, dodecyl ester	2.51	1678
11	14.185	Tetradecanoic acid, ethyl ester	2.57	1762
12	15.19	Ethyl oleate	2.45	1893
13	17.43	Hexadecanoic acid, methyl ester	3.19	1968
14	18.16	Phenol, 2,3,5-Trimethylphenol	15.92	1984
15	18.28	S-[2-[N,N-Dimethylamino]ethyl]N,N-dimethylcarbamoyl thiocarbohydroximate	4.22	2015
16	18.60	1-isothiocyanato-4-[methylsulfinyl] butane	20.89	2098
17	19.24	Octadecanoic acid	4.03	2100
18	22.90	Hexadecane	3.16	2896
19	23.73	Stigmasterol	2.02	3265
20	25.82	Ergost-5-en-3-ol	4.24	3240
21	26.90	gamma-Sitosterol	6.47	3334
Total			**100**	

Table 2. Differences between liver and kidney biochemical parameters in the studied groups with liver and kidney I/R.

	C	I/R-L	I/R-LB	I/R-LC
AST U/L	72.00±4.546a	228.5±8.693b	151.00±4.203c	184.25±3.065b
ALT U/L	39.75±4.605a	135.75±27.044b	123.50±1.190b	129.5±.645b
ALP U/L	63.50±1.322 a	94.5±2.397 a	195.17±18.754 a	86.25±1.376a
Creatinine mg/dL	0.51±0.031a	1.72±0.085b	0.91±0.016c	1.27±0.047d
Urea mg/dL	15.50±1.554a	43.50±2.179b	23.50±1.322 c	29.50±0.645c

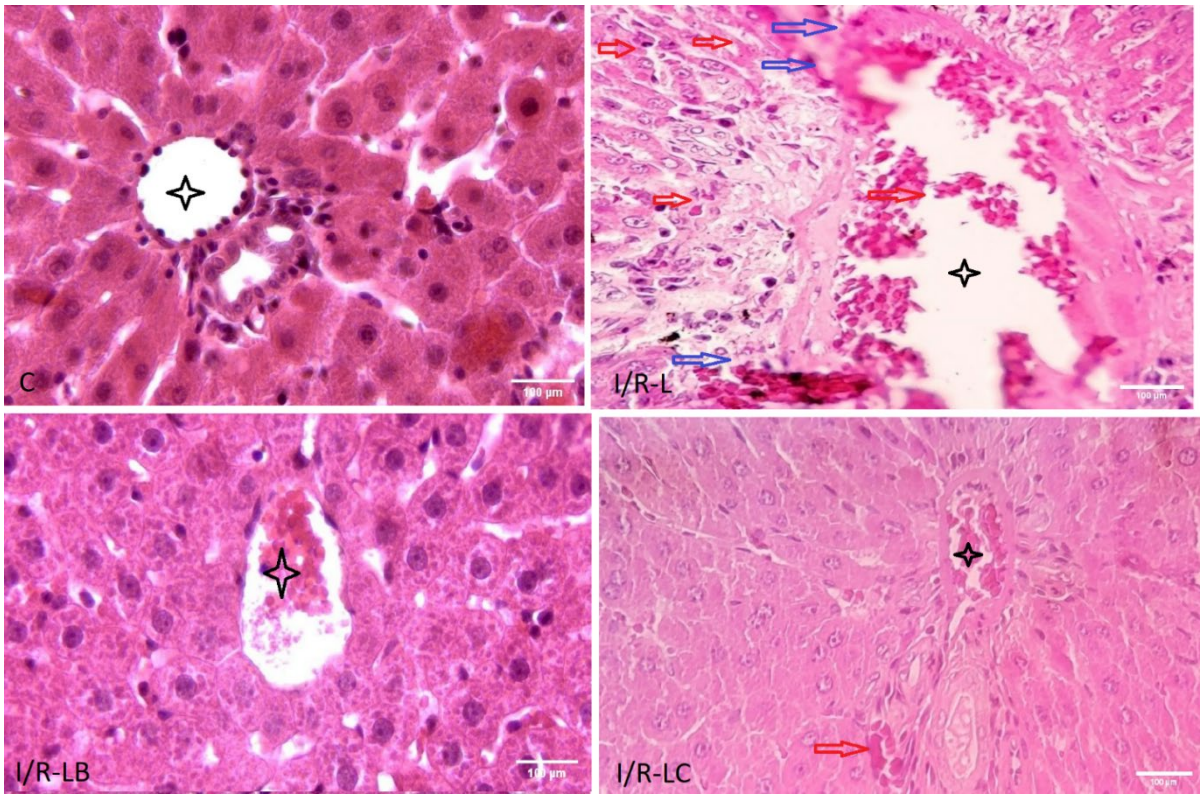

Figure 1. Histopathological changes in liver tissue following ischemia/reperfusion (I/R) injury. (Central vein indicated by star; inflammatory cells by red arrow; hemorrhage by blue arrow; H&E, ×400.) (**Prepared by Authors, 2026**).

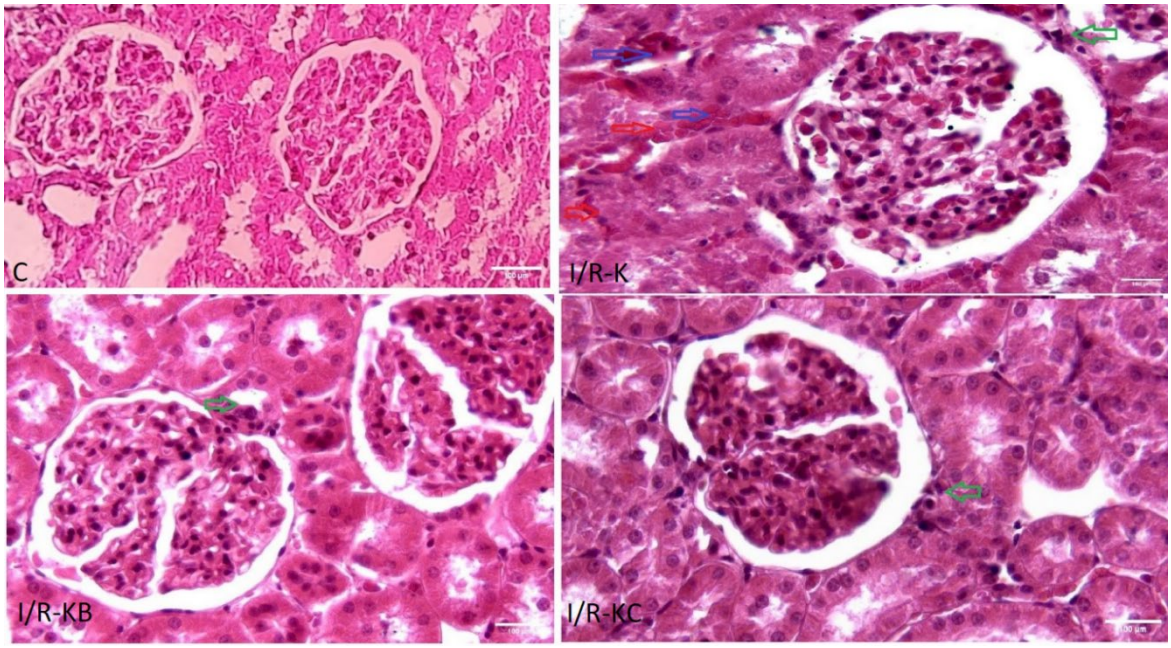

Figure 2. Histopathological changes in kidney tissue across experimental groups. (Hyperemia indicated in red, hemorrhage in blue, and inflammatory infiltration in green; H&E, ×400) (**Prepared by Authors, 2026**).

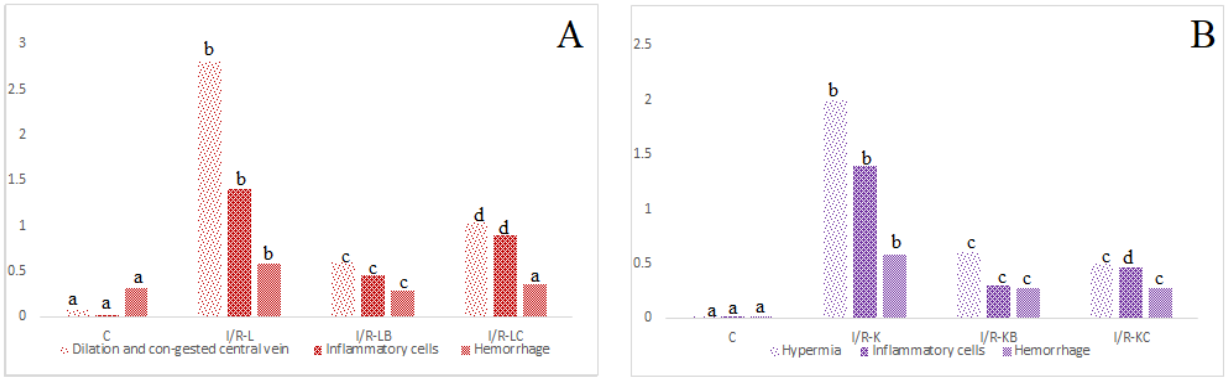

Figure 3. Histopathological grading of liver and kidney tissues in experimental groups. Distinct superscript letters (a–d) indicate statistically significant differences among groups ($p < 0.05$) (**Prepared by Authors, 2026**).

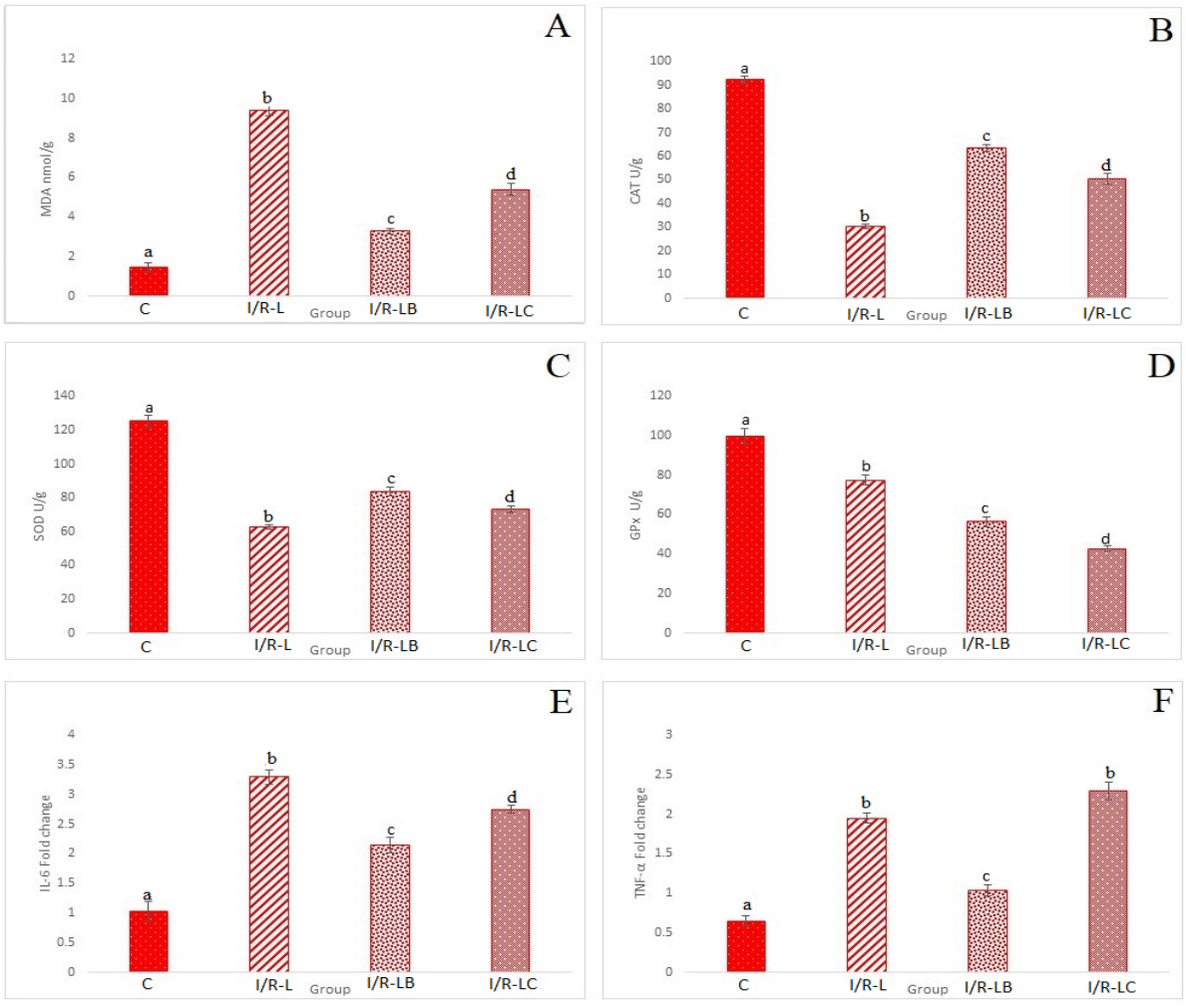

Figure 4. Liver oxidative stress markers and pro-inflammatory gene expression across experimental groups. Distinct superscript letters (a–d) indicate statistically significant differences ($p < 0.05$); MDA, malondialdehyde; CAT, catalase; SOD, superoxide dismutase; GPx, glutathione peroxidase; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha (**Prepared by Authors, 2026**).

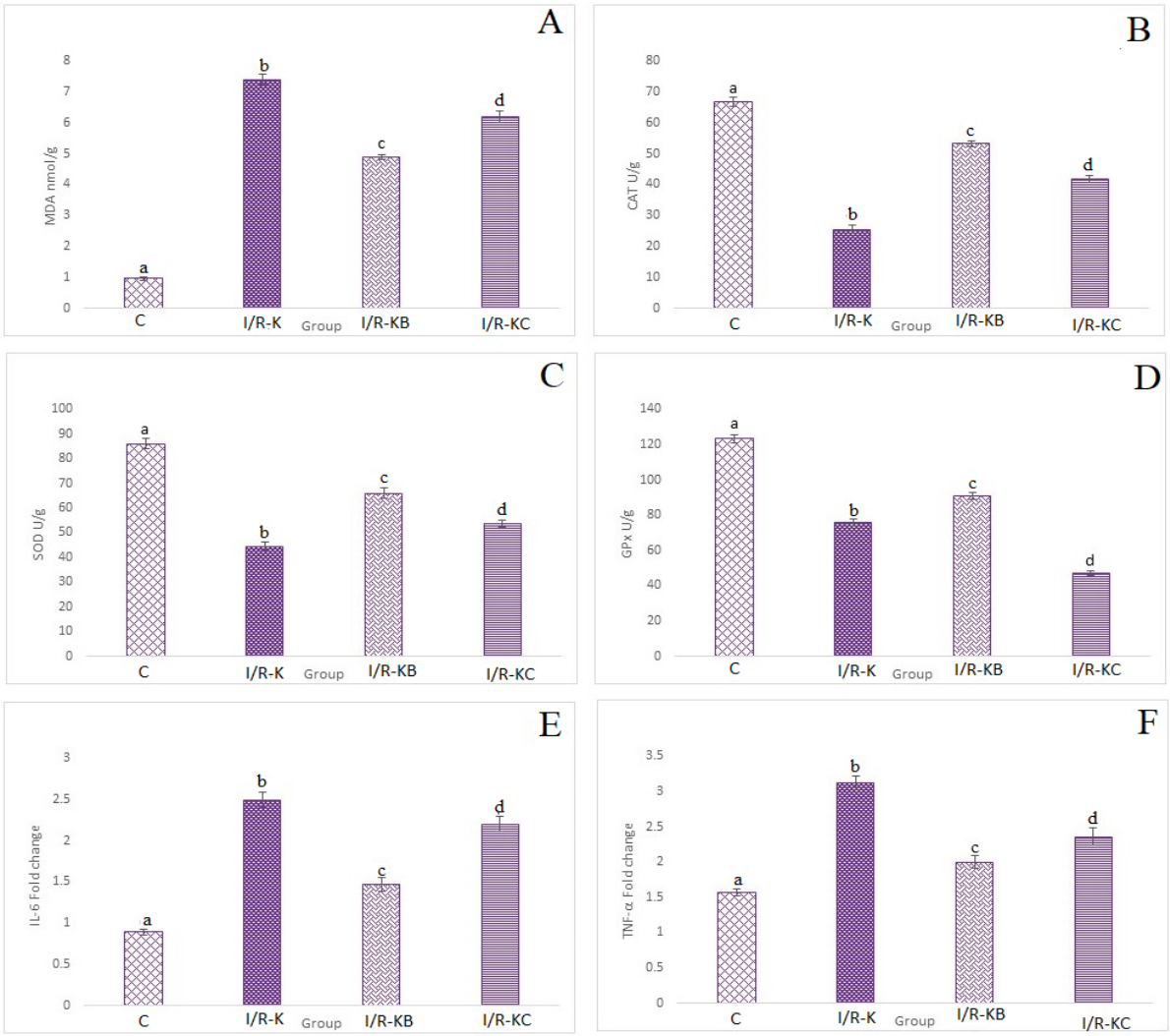

Figure 5. Kidney oxidative stress biomarkers and pro-inflammatory gene expression across experimental groups. Distinct superscript letters indicate statistically significant differences among groups ($p < 0.05$) (**Prepared by Authors, 2026**).

4. Discussion

The present findings indicate that pretreatment with broccoli extract provides more substantial protection against ischemia-reperfusion (I/R) injury in both the liver and kidneys compared with vitamin C. This enhanced effect is likely attributable to the combined action of its bioactive compounds, particularly sulforaphane, which plays a key role in attenuating oxidative stress and inflammatory responses during I/R injury. The observed elevations in serum AST, ALT, creatinine, and urea in the I/R groups confirm successful induction of the injury model and reflect typical cellular damage in hepatic and renal tissues following I/R **(28, 29)**.

The reduction of these biomarkers in the pretreated groups, especially with broccoli extract, suggests a direct protective effect on cell membrane integrity and organ function. These results are consistent with previous studies by Azari et al **(30)**, demonstrating that antioxidant combinations can mitigate renal I/R injury, and extend this concept by showing that a complex natural extract may outperform a single vitamin.

A notable aspect of the present study is the strong antioxidant potential of broccoli extract. The significant increase in tissue MDA levels in the I/R groups indicates severe lipid peroxidation and oxidative damage **(31)**. Pretreatment with broccoli effectively lowered MDA, demonstrating protection of lipid membranes. This effect is further supported by the extract's ability to maintain endogenous antioxidant enzyme activities, which constitute the primary defense against reactive oxygen species (ROS) **(32)**. During I/R, depletion of these enzymes exacerbates oxidative injury; broccoli extract, containing sulforaphane and phenolic compounds, not only scavenges free radicals but also enhances the native antioxidant system, providing an advantage over the single-action mechanism of vitamin C. These observations align with Raeeszadeh et al **(9)**, who reported superior antioxidant activity of broccoli

compared with vitamins C and E in a different experimental model.

At the molecular level, the increased mRNA expression of IL-6 and TNF-α in the I/R groups highlights the central role of inflammation in I/R injury (33). Reperfusion activates pro-inflammatory transcription factors, such as NF-κB (34). The significant reduction of these cytokines in the pretreated groups, particularly with broccoli extract, indicates a potent anti-inflammatory effect. This action is likely mediated by bioactive components of broccoli: sulforaphane activates Nrf2 and inhibits NF-κB, while phenolic compounds modulate pro-inflammatory mediators (35, 36). The superior protection conferred by broccoli extract can thus be attributed to sulforaphane-induced Nrf2 activation, which upregulates multiple endogenous antioxidant genes, providing broader defense compared with the direct scavenging effect of vitamin C (37, 38). These findings are consistent with Unsal et al (39), who reported that antioxidants can mitigate renal I/R injury by reducing inflammation, and further suggest that broccoli extract is particularly effective in this context.

Histopathological analyses reinforce the biochemical and molecular results. Reduced hemorrhage, congestion, and inflammatory infiltration in broccoli-pretreated groups visually confirm its protective role. The liver and kidney tissue architecture in the broccoli group was closest to normal and outperformed vitamin C, supporting its superior multi-faceted protective effect. Notably, liver tissue appeared more susceptible to oxidative damage, as reflected by higher MDA levels compared with kidney tissue, likely due to its higher lipid content (31). Nevertheless, broccoli extract provided effective protection in both organs, highlighting its broad therapeutic potential.

5. Conclusion

This study demonstrates that pretreatment with broccoli extract provides superior protection against ischemia-reperfusion (I/R) injury in the liver and kidneys compared with vitamin C. Broccoli extract more effectively prevents elevations in critical biomarkers, including AST, ALT, creatinine, and urea, indicating enhanced preservation of organ function. It also reduced malondialdehyde (MDA) levels and increased the activities of endogenous antioxidant enzymes, reflecting strengthened antioxidant defense. Furthermore, broccoli extract more effectively suppressed pro-inflammatory cytokines such as IL-6 and TNF-α, targeting a major mechanism of I/R injury. Histopathological analysis confirmed reduced cellular damage, hemorrhage, and inflammatory infiltration in broccoli-pretreated groups. The superior efficacy of broccoli extract is likely attributable to the synergistic activity of multiple bioactive compounds, particularly sulforaphane, which provides a multi-targeted protective mechanism compared with the single-action effect of vitamin C. These results suggest that broccoli extract is a promising therapeutic agent for preventing I/R injury in clinical settings, including organ transplantation and major surgical procedures. However, this study is limited by the evaluation of a single dose and a fixed treatment duration, and the long-term protective effects of the extract were not assessed. Future studies should investigate optimal dosing regimens, varying treatment schedules, and the sustained efficacy of broccoli extract to better define its clinical applicability and translational potential.

6. Declarations

6.1 Acknowledgments

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6.2 Ethical Considerations

All experimental procedures were approved by the Ethics Committee of Islamic Azad University of Sanandaj (IR.IAU.SDJ.REC.1400.074)

6.3 Authors' Contributions

All authors reviewed, edited, and approved the final version of the manuscript.

6.4 Conflict of Interest

The authors declare no conflict of interest.

6.5 Fund or Financial Support

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6.6 Using Artificial Intelligence Tools (AI Tools)

The authors were not utilized AI Tools.

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