The Protective Effects of Indole-Acetic Acid on the Renal Ischemia-Reperfusion Injury via Antioxidant and Anti-apoptotic Properties in A Rat Model

Sajad Papi,[1] Hassan Ahmadvand,[2,3]
Fattah Sotoodehnejadnematalahi,[4] Parichehreh Yaghmaei[1]

[1]Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2]Razi Herbal Medicine Research Center, Faculty of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran
[3]Department of Biochemistry, Faculty of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran
[4]Department of Biology, School of Basic Science, Science and Research Branch, Islamic Azad University, Tehran, Iran

Keywords. indoleacetic acid, renal ischemia-reperfusion injury, apoptosis, gene expression, oxidative stress

Introduction. The significant role of oxidative stress in the occurrence and development of a variety of diseases, including renal ischemia-reperfusion injury, has been thoroughly studied in this research. In this study, the protective role of indole-acetic acid on antioxidant, apoptotic and histopathological parameters in a rat model of renal ischemia-reperfusion (IR) injury were investigated.
Methods. We divided 40 rats into the following four groups (n = 10 per group): healthy control, IR control, IR + indole-acetic acid 40 mg/kg, and IR + indole-acetic acid 60 mg/kg. After two weeks, the rats were anesthetized and their kidneys were removed. The effects of indole-acetic acid on biochemical parameters [glutathione peroxidase (GPx) and catalase (CAT) were measured by spectrophotometry and expression of apoptotic genes (BAX and Bcl2) using real-time RT-PCR. Tubular necrosis was evaluated using a histopathological study.
Results. There were significant improvements in biochemical parameters (GPx), expression of the apoptotic genes (BAX) and tubular necrosis in rats treated with indole-acetic acid.
Conclusion. Indole-acetic acid could reduce the effects of factors involved in the pathogenesis of IR, including oxidative stress, apoptosis and tubular necrosis. It can be recommended that, indole-acetic acid may be useful for amelioration of damages caused by IR.

IJKD 2022;16:125-34
www.ijkd.org
DOI: 10.52547/ijkd.6894

Original Paper

INTRODUCTION

The high prevalence of chronic kidney disease (CKD) and end-stage kidney disease (ESKD) has become a challenging health problem with an extensive financial burden on the healthcare systems.[1] Identifying the risk factors associated with CKD and ESRD is key in reducing the incidence of this disease. Researchers have identified the role of acute kidney injury (AKI) as a risk factor for CKD.[2] Acute kidney injury is most commonly caused by the transient interruption of blood flow to the kidneys, which is known as renal ischemia-reperfusion injury (RIRI).[3] Several factors are involved in developmentof ischemia-reperfusion (IR), including kidney transplantation, nephrectomy, repair of suprarenal aneurysm, surgery, cardiac arrest, hemorrhagic shock, heart failure, trauma, and sepsis.[4] Ischemia- reperfusion occurs due to the temporary cessation of blood flow to the kidneys, followed by the resumption of the flow. The onset of ischemia, as the first phase of IR, is accompanied by the occurrence

of a reduction or an interruption of the entry of oxygen and nutrients into the kidney tissue. Production of toxic metabolites and reduction in cellular energy could be regarded as outcomes of renal ischemia.[5] Moreover, ischemia can induce the disruption of cellular metabolism. That is, the conversion of aerobic to anaerobic metabolism and ultimately leads to the induction of oxidative stress through the production of free radicals, including reactive oxygen species (ROS).[6] In the next phase of IR, reperfusion begins with restoration of blood flow and reoxygenation of kidney tissue. This phenomenon doubles the post-ischemia damage to the kidneys through activation of neutrophils, and production of ROS, inflammatory cytokines and chemokines.[7] Researchers have found that IR can be involved in the induction of renal cell apoptosis directly or indirectly through the production of ROS.[8] The role of ROS in the occurrence of IR has been proven.[9] They can damage cellular components by targeting biomolecules such as proteins and lipids and are also highly toxic to cellular components, particularly cell membranes, which are predominantly composed of lipids. Reactive oxygen species breakdown membrane lipids and creates lipid peroxides that are highly toxic to cells. They are metabolized via various non-enzymatic and enzymatic pathways to reduce oxidative stress within the cells. Hence, the use of antioxidants is efficient in the neutralization of ROS that are central to the development of IR.[10,11]

Researchers have long concentrated on the use of plant-derived antioxidants in the treatment of various diseases. These compounds have been widely used due to their natural origin, fewer side effects and greater efficacy.[12] Alkaloids are one of the most significant groups of secondary metabolites with several therapeutic properties.[13] Indole-acetic acid (IAA) is a member of the indole family of alkaloids, which is a plant hormone and is the most abundant plant auxin. The effect of IAA on increasing antioxidant enzymes during difficult conditions in plants has been investigated.[14] Moreover, researchers have found small amounts of IAA in mammals. Indole-acetic acid is produced by body through the metabolism of tryptophan and is excreted in the urine.[15] Numerous biological roles have been reported for IAA, including anti-inflammatory, antioxidant, and anti-apoptotic properties.[16] It has been shown that IAA exerts its anti-inflammatory properties by acting as a prostaglandin D_2 (PGD_2) antagonist.[17] Furthermore, since PGD_2 is involved in apoptosis, IAA has an anti-apoptotic function, and with its antioxidant properties inhibits tumor protein P53 (P53) by neutralizing free radicals and subsequently inhibiting the induction of apoptosis. It has also been reported that it plays an efficient anti-apoptotic role in activating B-cell lymphoma 2 (Bcl2) gene and inhibiting the caspase cascade by reducing Bcl-2-associated X protein (BAX) synthesis and activating the ATF factor.[18] Since inflammation and oxidative stress are two significant factors in inducing apoptosis and cellular cell necrosis, we investigated the antioxidant, anti-apoptotic and the ameliorating effects of IAA on tubular cell necrosis in a rat model of IR.

MATERIALS AND METHODS

Ethical Approval

The Animal Ethics Committee of Lorestan University of Medical Sciences approved all the experimental protocols.

Chemicals

The following kits and chemicals were used in this study: biochemical kits (Pars Azmoon Company, Iran) and indole-acetic acid, Glutathione, trichloric acid (TCA), tris-EDTA, dinitrothiocyanobenzene (DTNB), and phosphate-buffered saline (PBS) (all from Merck Company, Germany).

Animals and Study Design

The experimental procedures were carried out at Razi Herbal Medicines Research Center, affiliated to Lorestan University of Medical Sciences, Khorramabad, Iran. A total of 40 adult male Wistar rats (two months old; weight: 220 ± 10 g) were purchased and kept in standard cages. The rats were housed under appropriate environmental conditions including a temperature of 22 ℃, 12 hours light-dark cycles, and with free access to standard laboratory food and tap water. Razi Institutional Animal Care Committee at Lorestan University of Medical Sciences confirmed all of the study protocols and experimental procedures. The rats were randomly divided into four groups (10 rats per group): control (healthy) rats without IR surgery (group 1); control (IR) rats without receiving any treatment (group 2); IR + IAA rats

receiving 40 mg/kg oral IAA daily for two weeks (group 3), and IR + IAA rats receiving 60 mg/kg oral IAA daily for two weeks (group 4).

Induction of RIRI Model

Two weeks after pretreatment with IAA, the rats were fasted for 8 hours for IR surgery. Subsequently, the rats were anesthetized via intraperitoneal injection of thiopental (60 mg/kg). After anesthesia, the abdominal surface was shaved and sterilized using the povidone-iodine solution. The abdominal cavity was then exposed using a midline incision. Then, the location of the kidneys and renal pedicles was determined in the abdominal cavity, and their arteries were clamped for 45 minutes without damaging the arteries. Pale-color kidneys were considered as evidence of the ischemic phase. During ischemia, the abdominal cavity was covered with warm and humid sterile gauzes. After 45 minutes, the clamps were removed and blood flow to the kidneys was restored and the reperfusion phase began. The abdomens of the rats were then sutured and the rats were placed in the reperfusion phase for 24 hours.

Sample Collection

Twenty-four hours after the onset of the reperfusion, the rats were anesthetized with intraperitoneal injection of thiopental (60 mg/kg). Then, 5 mL of blood samples were directly collected from the hearts of the rats. The blood samples were kept in the laboratory for 20 minutes to allow for clot formation after which they were centrifuged at 3000 rpm for 15 min. The sera were isolated and kept at -70 °C till the biochemical measurements. The rats' kidneys were surgically removed. One of the kidneys was frozen in liquid nitrogen, and stored at -80 °C until gene expression studies. Another one was kept in 10% formalin solution (Histological) for histopathologicalevaluations.[19]

Biochemical Analysis

Measurement of Catalase (CAT). Catalase (CAT) activity (the conversion rate of H2O2 to H2O) was measured using a modified version of the method proposed by Aebi *et al.* Accordingly, the catalase converts H_2O_2 to H_2O and the activity of the catalase is measured based on the consumption of H_2O_2 and its reduced absorbance at 240 nm wavelength. For this purpose, 1 mL of 50 mM potassium phosphate (pH: 8) was added to 50 µL of the sample, followed by the addition of 50 µL H_2O_2. We used a spectrophotometer to measure the absorbance of sample in triplicate at 240 nm versus a blank for 0, 30, and 60 s. The absorbance was reported as U/mg.[20]

Measurement of Glutathione Peroxidase (GPx). Glutathione peroxidase (GPx) was measured using a modified version of the method proposed by Rotruck *et al.* In this method,the enzyme uses glutathione as the ultimate electron donor to regenerate the reduced form of the selenocysteine. By adding excess GSH, GPX converts it to GSSG and the remaining GSH can reduce and generate a yellow color by reducing DTNB. First, we added 200 µL of 0.4 M tris-HCl (pH 7), 100 µL of 1 mM NaN3, 200 µL of the sample, 200 µL of 2 mM glutathione, and 100 µL of 0.2 mM H_2O_2 into a tube, respectively. The tubes were kept at 37 °C for 10 min, after which we added 0.4 mL of 10% TCA to the tubes. The tubes were centrifuged at 2000 rpm for 3 min. A total of 25 µL of the supernatant was poured into an ELISA microplate where it was mixed with 140 µL of 0.2 M tris-EDTA (pH 8) and 30 µL of DTNB. After 30 min incubation at room temperature, an ELISA reader was used to calculate the absorbance of each sample in triplicate at 420 nm versus a blank. The GPx levels were reported as U/mg.Sodium citrate was used as the solvent for DTNB.[21]

Antioxidant Enzyme Gene Expression

RNA Extraction and Quantitative Real-Time PCR. Total RNA was extracted from kidney tissues using the TriPure RNA Isolation Reagent (Roche Applied Science, Germany) according to the manufacturer's instructions. Purified RNA was kept at -80 °C until use. Electrophoresis on an agarose gel was used to determine integrity of purified RNA. cDNA synthesis was carried out with 2.0 µg of the total RNA sample and oligo dT primers with an M-MuLV RT kit (MBI Fermentas, Lithuania) in a net reaction volume of 20 µL. The levels of mRNA levels for target genes (*Bax*, and *Bcl-2*) and the reference gene (*β-actin*) were analyzed in triplicate by quantitative real-time RT-PCR using ABI 7500 Sequence Detection System (Applied Biosystems, USA). For this purpose, initial pre-cycling thermal activation was carried out for 10 min at 95 °C. Then, 40 cycles of amplification was performed for

Table 1. Primers' Sequence Used for Quantitative RT-PCR

Gene	Forward Primer	Reverse Primer	Product Size (bp)
Bax (Gene ID: 116892149)	5′CGTGGTTGCCCTCTTCTACTTT3′	5′GATCAGCTCGGGCACTTTAGTG3′	73
Bcl-2 (Gene ID: 116913126)	5′GATGACTTCTCTCGTCGCTA3′	5′GTCATCCACAGAGAGCGATGTT3′	229
β-actin (Gene ID: 116895656)	5′CATCGGCTTGAGAAAAGGAG3′	5′TATCGGCAATGAGCGGTTCC3′	150

as the following steps: denaturation at 95 °C for 15 s, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min. Ultimately, the period of the final extension reaction was increased to 10 min at 72 °C for the fulfillment of reaction products. Table 1 shows the primer sequences.[22]

Histopathological Evaluation

PeriodicAcid-Schiff (PAS) staining was used to evaluate tubular necrosis. About 40 fields of view from each kidney specimen with 400× magnification were examined for detection of tubular necrosis, leukocyte infiltration, and eosinophilic casts using Nikon model light microscope. Leukocyte infiltration was assessed using the Velasquez method. Tubular necrosis was assessed using the Caramelo method.[23]

Statistical Analysis

The biochemical and histopathological results were calculated as Mean ± SD and the gene expression results as Mean ± SEM. We used SPSS 18 software for statistical analyses. Data analysis was performed using one-way ANOVA (Tukey's multiple comparison test). $P < .05$ indicated statistical significance.

RESULTS

Effects of IAA on Serum Catalase (CAT) Levels

Our findings showed significant reduction in serum CAT levels (nmol/minute/mg protein) in the control (IR) rats compared with control (healthy) rats ($P < .05$). Nevertheless, there were no significant changes in serum CAT levels in the groups treated with IAA at a dose of 40 mg/kg and 60 mg/kg (Figure 1).

Effects of IAA on Serum Glutathione Peroxidase (GPx) Levels

As shown in Figure 2, the induction of IR significantly decreased GPx (µmol/mg protein) levels in the control (IR) rats compared to the control (healthy) rats ($P < .05$). Administration of IAA in the IR + IAA 60 mg/kg group significantly increased GPx levels compared to the control (IR) group ($P < .05$). Nonetheless, there were no significant difference in serum GPx levels between the IR + IAA 40 mg/kg group compared to the control (IR) group.

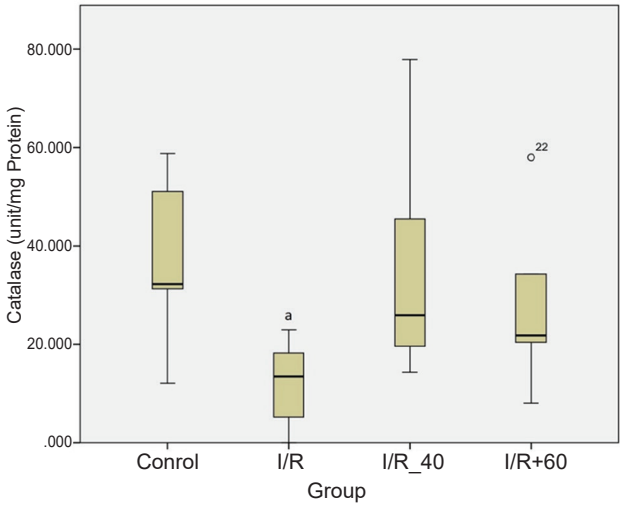

Figure 1. Effects of IAA on serum CAT levels in different groups (A: Shows a significant difference with the control (healthy) group ($P < .05$), B: shows a significant difference with the control (IR) group ($P < .05$)). Values have been expressed as mean ± SD.

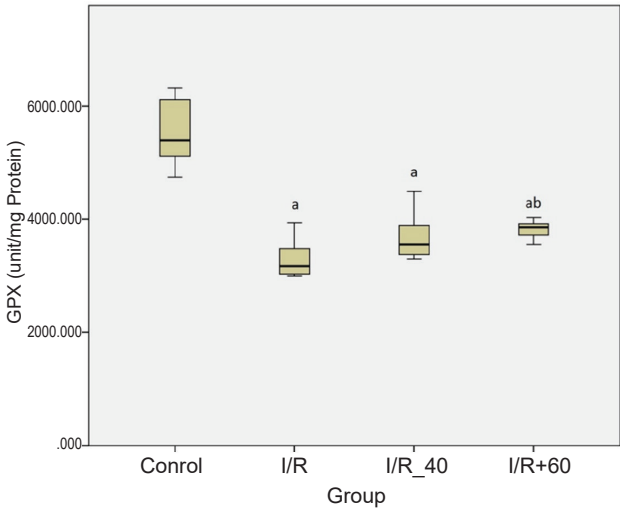

Figure 2. Effects of IAA on serum GPx levels in different groups (A: shows a significant difference with the control (healthy) group ($P < .05$), B: shows a significant difference with the control (IR) group ($P < .05$)). Values have been expressed as mean ± SD.

Effects of IAA on the Gene Expression of Bcl-2 in the Rats' Kidney Tissues

Figure 3 and Figure 4 show the fluctuations in the relative gene expression of Bcl-2 in the rat's kidney tissues.

Effects of IAA on the Gene Expression of Bax in the Rats' Kidney Tissues

As it has been shown in Figure 5 and Figure 6, the results showed that induction of IR could significantly increase the relative gene expression of Bax in the kidney tissues of the control (IR) rats compared to the control (healthy) rats ($P < .05$). Administration of IAA in the IR + IAA 40 mg/kg group significantly decreased the relative gene expression of Bax in comparison with the control (IR) group ($P < .05$). Moreover, the use of IAA at a dose of 60 mg/kg significantly decreased the relative gene expression of Bax in the rats of the IR + IAA 60 mg/kg group in comparison with the control (IR) rats ($P < .05$).

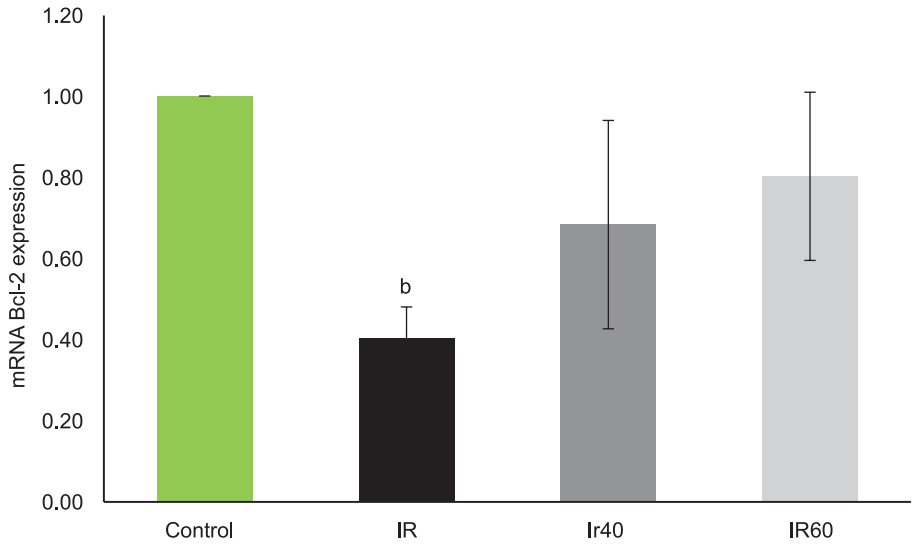

Figure 3. Effect of IAA on the relative gene expression of Bcl-2 in the rats' kidney tissues of different groups (A: shows a significant difference with the control (healthy) group ($P < .05$), B: shows a significant difference with the control (IR) group ($P < .05$)). Values have been expressed as mean ± SEM.

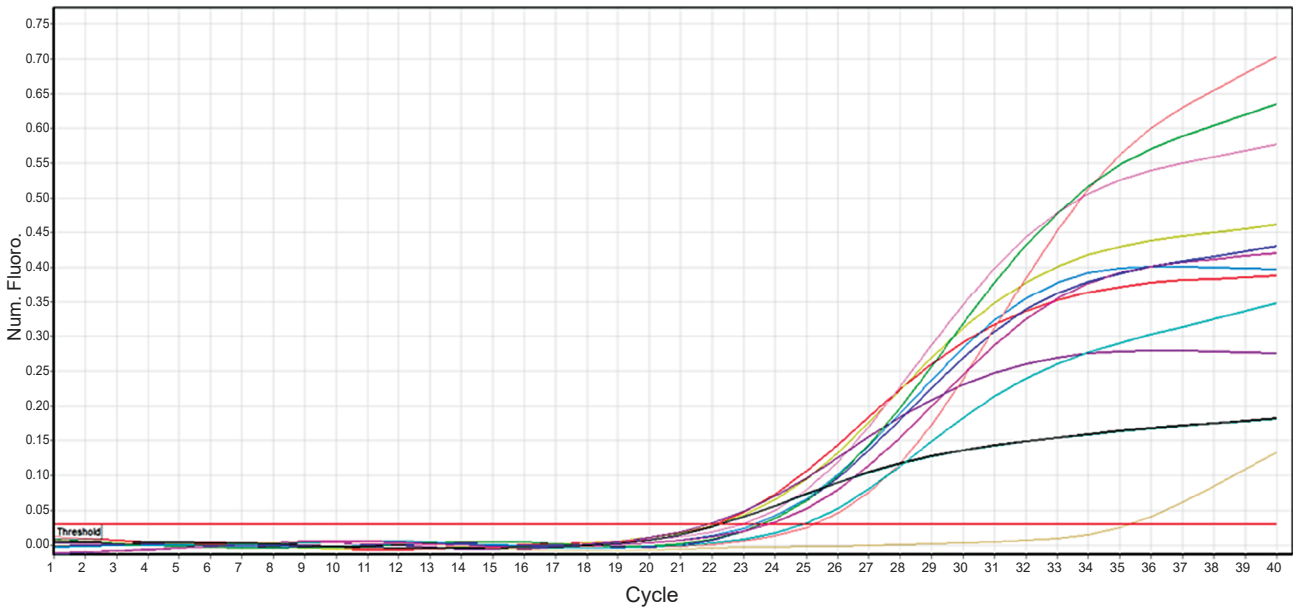

Figure 4. Amplification curve of Bcl-2 mRNA gene expression (fluorescence signal plotted versus cycle number).

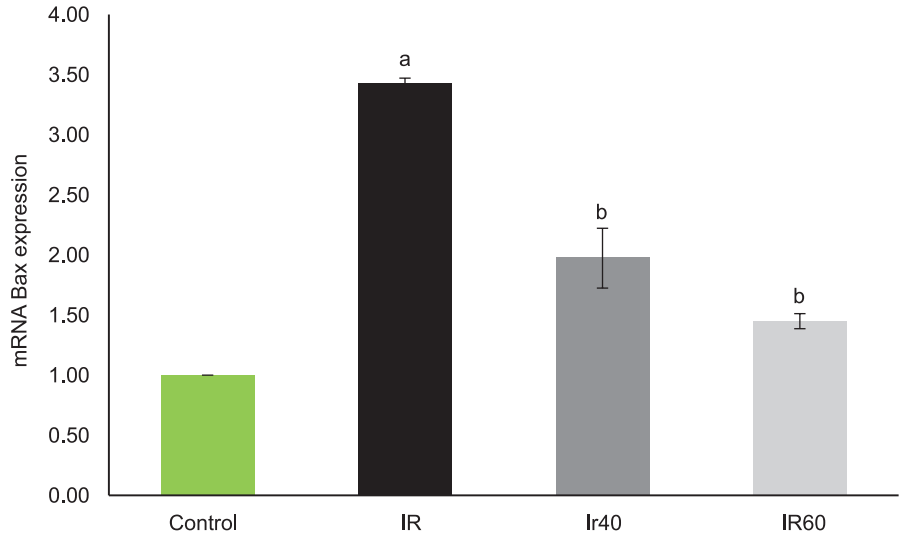

Figure 5. Effect of IAA on the relative gene expression of Bax in the rats' kidney tissues of different groups (A: shows a significant difference with the control (healthy) group ($P < .05$), B: shows a significant difference with the control (IR) group ($P < .05$)). Values have been expressed as mean ± SEM.

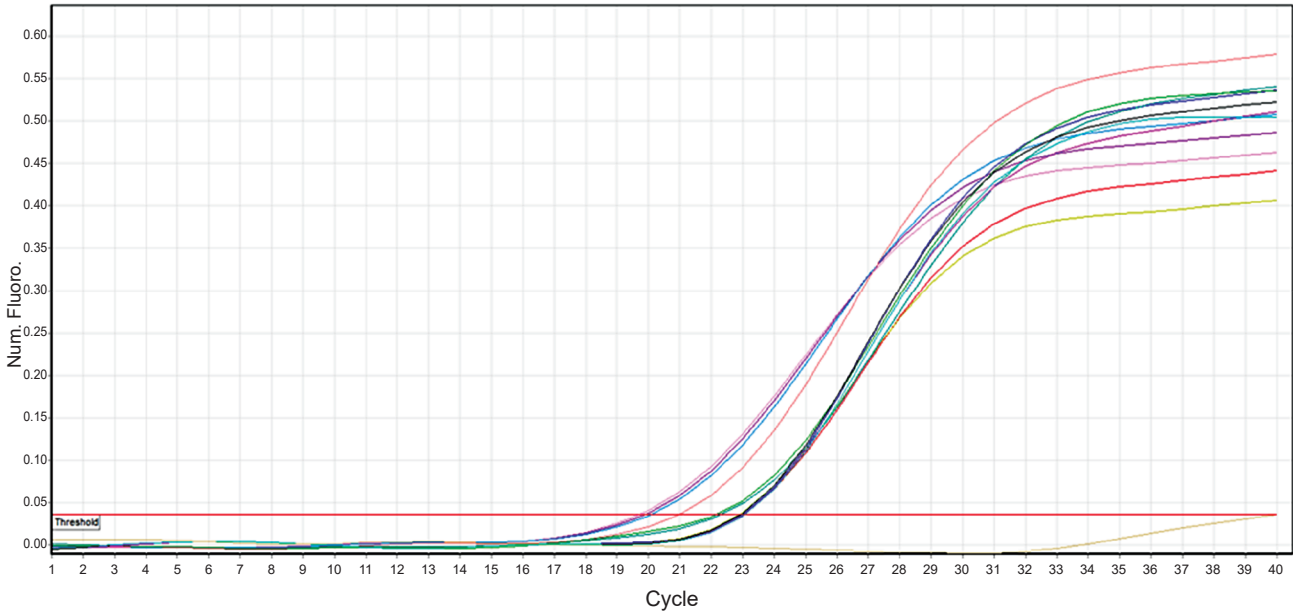

Figure 6. Amplification curve of Bax mRNA gene expression (fluorescence signal plotted versus cycle number).

Effects of IAA on Histopathological Parameters

Figure 7 exhibits the histopathological alterations in different groups. Figure 8 displays the tubular necrosisin different groups. It was revealed that induction of IR could significantly increase tubular necrosis in the kidney tissues of the control (IR) rats compared to the control (healthy) rats ($P < .05$). Administration of IAA in the IR + IAA 40 mg/kg group significantly decreased tubular necrosis compared to the control (IR) group ($P < .05$). Furthermore, the use of IAA at a dose of 60 mg/kg significantly decreased tubular necrosis in the rats of the IR + IAA 60 mg/kg group compared to the control (IR) rats ($P < .05$).

DISCUSSION

Ischemia-reperfusion is considered as one of the most significant causes of CKD. It can eventually lead to irreversible complications such as ESKD. Oxidative stress and the production of ROS have definite roles in the development of IR-induced damage. Researchers have found that ROS can be involved

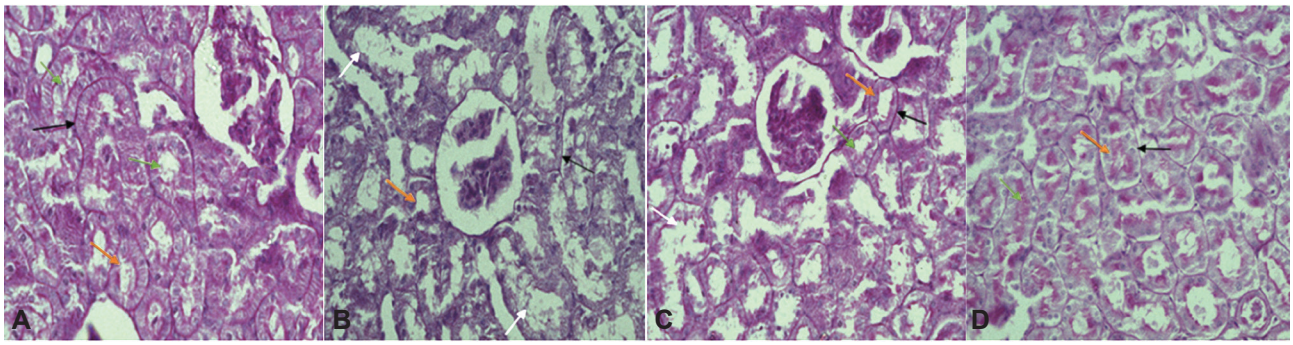

Figure 7. Effects of IAA on histopathological parameters in different groups (Photomicrographs of rats' renal tissue: A) micrograph from the kidney of a rat in the sham group showed normal tubules and brush border, B) micrograph from the kidney of a rat in the I/R group showed tubular necrosis without brush border, C) micrograph from the kidney of a rat in the IR + IAA 40 mg/kg group showed improvement in tubular necrosis and brush border compared with I/R group, D) micrograph from the kidney of a rat in the IR + IAA 60 mg/kg group showed improvement in tubular necrosis and brush border compared with I/R group).
Black arrow is showed PAS reaction along the basement membrane. Green arrow is showed brush border of renal tubules. Orange arrow is showed normal histological structure of renal tubular. Gray arrow is showed renal epithelial tubular necrosis.

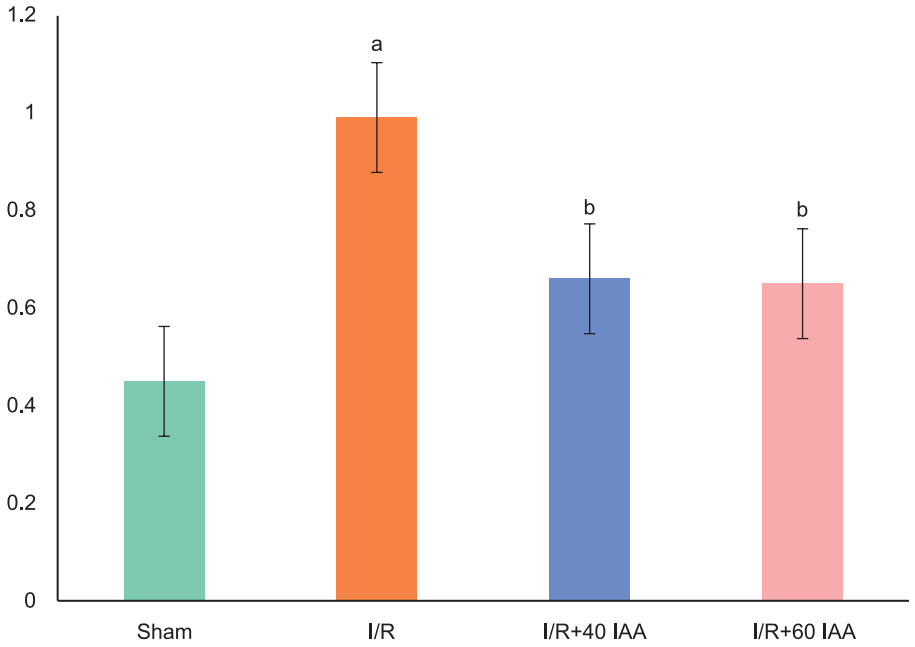

Figure 8. Effects of IAA on tubular necrosis in different groups (A: shows a significant difference with the control (healthy) group ($P < .05$), B: shows a significant difference with the control (IR) group ($P < .05$)). Values have been expressed as mean ± SD.

in the induction of apoptotic and inflammatory cascades. Hence, antioxidant compounds can be proposed as a good solution for scavenging ROS and preventing the development of IR-induced damage.[5] Therefore, the aim of this study was to assess whether IAA could protect the kidneys from IR by its anti-oxidative and anti-apoptotic activities. This study was the first research which investigated the protective effect of IAA on the damage caused by IR through its antioxidant and anti-apoptotic effects. Another aspect of our findings indicated that IR could suppress antioxidant enzymes, including Gpx and CAT. Furthermore, administration of IAA reversed this effect, particularly in CAT in which this agent was accompanied by a significant elevation in enzyme levels. The underlying mechanism of this elevation could be related to the ability of IAA in scavenging ROS.[24] Another proposed mechanism is that the IAA activates the transcription factor nuclear factor erythroid 2–related factor 2 (Nrf2), which promotes the expression and enhancement of antioxidant enzymes that ultimately reduce oxidant damage and cell death.[25] A similar study assessed the effect of IAA on oxidative stress caused

by H_2O_2 in human stem cells. It was revealed that IAA administration could protect stem cells against oxidative stress induced by H_2O_2 through enhanced expression of Nrf2 and heme oxygenase 1 (HO-1), which involved the AKT pathway.[18] In yetanother study the effect of IAA on nonalcoholic fatty liver diseasein mice was evaluated. The results of this study indicated that IAA treatment could restore the markers of oxidative stress, including ROS, MDA, GSHand SOD. In fact, antioxidant compounds such as indole-3-acetic acid could reduce the damage to kidney tissue and the membranes of renal cells by reducing oxidative stress-related lipid peroxidation, particularly MDA, which in turn reduces the consumption of antioxidant enzymes scavengingfree radicals. Moreover, indole-3-acetic acid participates in prevention of the consumption of antioxidant enzymes such as CAT, Gpx and SOD in cells by restoring antioxidant stores such as glutathione.[26] The results of the studies mentioned above were consistent with the findings of our study. During IR, oxidation of lipids, proteins, and nucleic acids cause damage. Researchers have shown that production of ROS during IR plays a central role in the development of inflammatory and apoptosis cascades, and ultimately cellular and tissue damage.[27] Our findings indicated that induction of IR could significantly enhance the relative gene expression of Bax in the kidney of the rats. Furthermore, pretreatment with the IAA could significantly suppress relative gene expression of Bax. We also evaluated the relative expression of another apoptotic gene Bcl-2 which had no significant fluctuation. Apoptosis begins as a result of an imbalance between two apoptosis-inducing proteins called BAX and an apoptosis-inhibiting protein called Bcl-2. Researchers have shown that during the ischemic process, the tissue becomes oxygen deficient. This lack of oxygen causes the apoptotic activating proteins to be released from the cytoplasm and travel to the mitochondrial membrane, causing changes in the outer mitochondrial membrane. Subsequently, cytochrome C is released and the apoptotic process begins. Unlike BAX, Bcl2 prevents rupture and changes in the mitochondrial membrane and acts as an inhibitor of apoptosis.[28,29] Researchers have shown that IAA plays a significant role in reducing BAX protein expression by decreasing oxidative stress. The IAA can also be useful in inducing Bcl2 protein expression and inhibiting apoptosis by triggering certain activating factors. Moreover, it has been indicated that alkaloid compounds can be highly efficient in reducing IR damage by inhibiting BAX protein expression and inducing Bcl2 protein expression.[29]

Furthermore, evaluation of histopathological parameters revealed that induction of IR could significantly elevate tubular necrosis in the kidney tissue rats. It has been demonstrated that administration of IAA significantly decreases tubular necrosis. Researchers have reported that damage caused by IR is related to tubular necrosis and apoptosis. Certain events occur during necrosis, including cell lysis, protein denaturation, and activation of inflammatory factor receptors. It has been shown that IAA has an anti-inflammatory role as a prostaglandin inhibitor.[17]. In this study, IAA with its antioxidant and anti-inflammatory properties reduced oxidative damage and tubular necrosis. It has been established that IAA alleviates the production of pro-inflammatory cytokines from macrophages.

CONCLUSION

The results of the this study showed that indole-acetic acid can attenuate the factors involved in the pathogenesis of IR, including oxidative stress, apoptosis and tubular necrosis. Furthermore, pretreatment with IAA enhanced the levels of antioxidant enzymes. Moreover, IAA may affect the apoptosis process through increased expression of Bcl2 and decreased expression of BAX.On the other hand, IAA improved tubular necrosis. Hence, the use of indole-acetic acid is suggested for the amelioration of damage caused by IR. Increasing the number of doses of IAA and evaluating the anti-inflammatory properties are advisable for further studies.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to the officials of Lorestan University of Medical Sciences (LUMS), Khorramabad, Iran for their support. We also thank the chairman and staff of Razi Herbal Medicines Research Center, affiliated to LUMS, for their financial support of this research.

AUTHORS' CONTRIBUTION

HA, FS designed the project. SP collected the data. HA analyzed the data. SP wrote the manuscript.

PY revised the English version and edited the final draft. All the authors signed the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL CONSIDERATION

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

FUNDING / SUPPORT

Funding was provided by Lorestan University of Medical Sciences.

REFERENCES

1. Wang, V., Vilme, H., Maciejewski, M. L., & Boulware, L. E. The economic burden of chronic kidney disease and end-stage renal disease.Semin Nephrol. 2016;36(4): 319-330.
2. Le Clef, N., Verhulst, A., D'Haese, P. C., & Vervaet, B. A. Unilateral renal ischemia-reperfusion as a robust model for acute to chronic kidney injury in mice. PloS one. 2016; 11(3): e0152153.
3. Sakai K, Nozaki Y, Murao Y, et al. Protective effect and mechanism of IL-10 on renal ischemia–reperfusion injury. Lab Invest. 2019; 99(5): 671-683.
4. Ahmadvand, H., Babaeenezhad, E., Nasri, M., Jafaripour, L., & Khorramabadi, R. M. Glutathione ameliorates liver markers, oxidative stress and inflammatory indices in rats with renal ischemia reperfusion injury.J Renal Inj Prev. 2018; 8(2): 91-97.
5. Ahmadvand H, Yalameha B, Adibhesami G, et al. The protective role of gallic acid pretreatment on renal ischemia-reperfusion injury in rats.Rep Biochem Mol Biol. 2019; 8(1): 42.
6. Ahmadvand, H., Babaeenezhad, E., Nayeri, H., & Nezhad, Z. Z. Selenium effects on antioxidant and inflammatory indices in renal ischemia-reperfusion injury in rats.J Renal Inj Prev. 2018; 8(2): 71-77.
7. Hasanvand, A., Abbaszadeh, A., Darabi, S., Nazari, A., Gholami, M., & Kharazmkia, A. Evaluation of selenium on kidney function following ischemic injury in rats; protective effects and antioxidant activity.J Renal Inj Prev.2017; 6(2): 93.
8. Gu Y, Huang F, Wang Y, et al. Connexin32 plays a crucial role in ROS-mediated endoplasmic reticulum stress apoptosis signaling pathway in ischemia reperfusion-induced acute kidney injury.J Transl Med.2018; 16(1): 1-13.
9. Mohmoodnia L, Koushki S, Moruzzi M, et al. Protective effect of aliskiren against renal ischemia reperfusion via antioxidant property and nitric oxide signaling pathway. Immunopathol Persa. 2019; 5(1): e10-e10.
10. Ahmadvand H, and Mahdavifard S. Protective effect of thioctic acid on renal ischemia–reperfusion injury in rat.Int J Prev Med. 2019; 10:176.
11. Garrel C, Fowler P A, and Al-Gubory K H. Developmental changes in antioxidant enzymatic defences against oxidative stress in sheep placentomes.J Endocrinol. 2010; 205(1): 107-116.
12. Cui X, Lin Q, and Liang Y. Plant-Derived Antioxidants Protect the Nervous System From Aging by Inhibiting Oxidative Stress.Front Aging Neurosci. 2020; 12: 209.
13. Roy, A. A review on the alkaloids an important therapeutic compound from plants. IJPB. 2017; 3(2): 1-9.
14. Sadauskas, M., Statkevičiūtė, R., Vaitekūnas, J., & Meškys, R. Bioconversion of Biologically Active Indole Derivatives with Indole-3-Acetic Acid-Degrading Enzymes from Caballeronia glathei DSM50014. Biomolecules.2020; 10(4): 663.
15. Konopelski, P., Konop, M., Gawrys-Kopczynska, M., Podsadni, P., Szczepanska, A., & Ufnal, M. Indole-3-propionic acid, a tryptophan-derived bacterial metabolite, reduces weight gain in rats. Nutrients. 2019; 11(3): 591.
16. Ji, Y., Yin, W., Liang, Y., Sun, L., Yin, Y., & Zhang, W. Anti-Inflammatory and Anti-Oxidative Activity of Indole-3-Acetic Acid Involves Induction of HO-1 and Neutralization of Free Radicals in RAW264. 7 Cells.Int J Mol Sci.2020; 21(5): 1579.
17. Armer R E, Ashton M R, Boyd E A, et al. Indole-3-acetic acid antagonists of the prostaglandin D2 receptor CRTH2.J Med Chem.2005; 48(20): 6174-6177.
18. Kim, D., Kim, H., Kim, K., & Roh, S. The protective effect of indole-3-acetic acid (IAA) on H2O2-damaged human dental pulp stem cells is mediated by the AKT pathway and involves increased expression of the transcription factor nuclear factor-erythroid 2-related factor 2 (Nrf2) and its downstream target heme oxygenase 1 (HO-1).Oxid Med Cell Longev.2017; 2017: 8639485.
19. Tavafi, M., Ahmadvand, H., Tamjidipour, A., & Hasanvand, A. Rosmarinic acid ameliorates renal ischemia reperfusion damage in rats.J Nephropharmacol. 2020; 9(2): e15.
20. Nasiry, D., Khalatbary, A. R., Ahmadvand, H., & Talebpour Amiri, F. B. Effects of Juglans regia L. leaf extract supplementation on testicular functions in diabetic rats. Biotech Histochem. 2021; 96(1):41-47.
21. Nasri, M., Adibhesami, G., Mahdavifard, S., Babaeenezhad, E., & Ahmadvand, H. Exogenous glutamine ameliorates diabetic nephropathy in a rat model of type 2 diabetes mellitus through its antioxidant and anti-inflammatory activities.Arch Physiol Biochem. 2020; 1-10.
22. Jamor, P., Ahmadvand, H., Ashoory, H., & Babaeenezhad, E. Effect of alpha-lipoic acid on antioxidant gene expression and kidney injury in alloxan-induced diabetic rats.J Nephropathol.2019; 8(1).
23. Ahmadvand, H., Shahsavari, G., Tavafi, M., et al. Protective effects of oleuropein against renal injury oxidative damage in alloxan-induced diabetic rats; a histological and biochemical study.J Nephropathol. 2017; 6(3): 204.
24. Khan, M. Y., Prakash, V., Yadav, V., et al. Regulation of cadmium toxicity in roots of tomato by indole acetic acid with special emphasis on reactive oxygen species production and their scavenging.Plant Physiol Biochem. 2019; 142: 193-201.

25. Martin, D., Rojo, A. I., Salinas, M., et al. Regulation of heme oxygenase-1 expression through the phosphatidylinositol 3-kinase/Akt pathway and the Nrf2 transcription factor in response to the antioxidant phytochemical carnosol.J Biol Chem.2004; 279(10):8919-29.

26. Oliveira, D. L., Pugine, S. M. P., Ferreira, M. S. L., Lins, P. G., Costa, E. J. X., & de Melo, M. P. Influence of indole acetic acid on antioxidant levels and enzyme activities of glucose metabolism in rat liver.Cell Biochem Funct.2007;25(2):195-201.

27. Redza-Dutordoir, M., and Averill-Bates, D. A. Activation of apoptosis signalling pathways by reactive oxygen species. Biochim Biophys Acta. 2016;1863(12):2977-2992.

28. Burns, A. T., Davies, D. R., Mclaren, A. J., Cerundolo, L., Morris, P. J., & Fuggle, S. V. Apoptosis in ischemia/reperfusion injury of human renal allografts1. Transplantation. 1998;66(7):872-6.

29. Zheng, H., Lan, J., Li, J., & Lv, L. Therapeutic effect of berberine on renal ischemia-reperfusion injury in rats and its effect on Bax and Bcl-2.Exp Ther Med. 2018;16(3):2008-2012.

Correspondence to:
Hassan Ahmadvand, MD
Department of Biochemistry, Faculty of Medicine, Lorestan University of Medical Sciences, Post Code: 6813833946, Khorramabad, Iran
Tel: 009 66 3330 0661
E-mail: hassan_a46@yahoo.com