Protective Effects of Alpha-Pinene Pre-Treatment in Renal Ischemia-Reperfusion Injury in Male Rats

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Keywords. Alpha-pinene; Renal ischemia-reperfusion; Oxidative stress; Inflammatory cytokines; Apoptosis

Introduction. Ischemia followed by reperfusion in organ transplantations can lead to ischemia-reperfusion (I-R) injury, which is associated with oxidative stress and inflammatory responses. Alpha-pinene is an organic terpene with well-known antioxidant, anti-inflammatory, and anti-apoptotic properties. This study examines the preventive effects of alpha-pinene against renal I-R-induced kidney dysfunction, oxidative and inflammatory status, apoptosis, and histopathology changes.

Methods. Forty-two adult male Wistar rats weighting 200-250 gr were divided into six groups (n = 7): Control, Right Nephrectomy, Ischemia-Reperfusion (45 min ischemia and 24 h reperfusion), and I-R + three different doses of alpha-pinene (2.5, 5, and 10 mg/kg) 24 hours and just before induction of ischemia through gavage. After 24 hours, urine, serum, and the remaining kidney were collected for biochemical and tissue analysis.

Results. Renal I-R caused kidney damage indicated by a significant decrease in creatinine clearance, induction of oxidative stress, increased inflammatory cytokines, and histopathological injuries. Alpha-pinene significantly improved the damage by restoring the changes toward the control group. Alpha-pinene, in the effective dose (2.5 mg/kg), reduced the levels of Bax, Bcl-2, TNF-α, and IL1β and contributed to regenerating tissue damage following renal I-R.

Conclusions. Aalpha-pinene has been able to reduce the complications due to its antioxidant, anti-inflammatory, and anti-apoptotic properties. It is suggested that it can be used as a pretreatment in reducing renal complications in renal transplantation.

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INTRODUCTION

Ischemia-reperfusion (I-R) is considered as the interruption of the blood supply to tissues for a limited period, followed by restoring the blood flow.[1] Acute kidney injury (AKI) is a common condition in hospitalized patients, which is usually caused by renal ischemia-reperfusion and can be observed in various situations such as cardiovascular surgery, kidney transplantation, and sepsis.[2–4] Reperfusion is necessary for ischemic tissues; however, it can result in some damages.[5] Reperfusion leads to re-oxygenation in ischemic tissues, generating excess amount of reactive oxygen species (ROS), ending in oxidative stress, inflammation, apoptotic cell death, and ultimately disturbances to the structure and function of the kidneys.[6–8]

Oxidative damage results in the formation of cytotoxic products such as malondialdehyde (MDA) due to lipid peroxidation.[9] Accumulation

of intracellular ROS accelerates inflammation and reduces levels of endogenous antioxidant enzymes, including glutathione peroxidase and catalase.[10] Following the initiation of reperfusion, a cascade of inflammatory responses is started. Reports indicate that NF-κB activation is involved in the initiating inflammation in I-R. Afterward, it stimulates the production of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin (IL-1, 6).[11,12] Incidence of apoptosis is a consequence of reperfusion. Therefore, suppression of inflammatory mediators and regulation of the expression of apoptotic proteins, such as Bax/Bcl-2, can effectively decrease the injury.[13,14] In order to reduce the I-R damage, various therapeutic approaches have been provided, and due to the overproduction of free radicals, the use of antioxidants can be useful to reduce the side effects.

Medicinal plants and their derivatives have been considered for their therapeutic properties.[15] Their essential oils, for example, with antioxidant and anti-inflammatory activities, have beneficial effects on oxidative stress caused by renal I-R injury.[16]

Terpenes are a group of compounds with various medicinal properties that are found in both plants and animals.[17] Alpha-pinene is a two-ring monoterpene found in the essential oils of conifers, including pine and other herbs, including rosemary, camphor, and eucalyptus.[18] Experiments have shown that alpha-pinene has various pharmacological effects such as antioxidative, analgesic,[19] antibacterial,[20] and anti-anxiety activities.[21] Borges *et al.* have recently demonstrated that alpha-pinene strongly inhibits inflammation in pathological conditions by inhibiting the production of inflammatory factors such as NF-κB.[22] Another study by Karthikeyana *et al.* revealed that it reduced apoptosis by regulating Bax/Bcl-2 expression.[23]

As injuries following organ transplantations are major issues, and additionally, there is a chance of rejection following these operations, discovering techniques to prevent these complications seems obligatory. Therefore, in this study, we assessed preventive properties of alpha-pinene by administering it twice before renal ischemia-reperfusion to simulate organ transplant condition.

Given that oxidative stress and inflammation play essential roles in I-R pathophysiology, we assessed inflammatory, apoptotic, oxidative, functional, and histopathological changes following renal I-R and preventive properties of alpha-pinene to alleviate the resulting disturbances.

MATERIALS AND METHODS

Animals and Experimental Design

Forty-two male Wistar rats (weighing 200-250g, 9-week-old) were purchased from the Animal Center of the Kerman University of Medical Sciences (Kerman, Iran). They were housed in standard conditions with a temperature of 23 ± 1°C under a 12 h light/dark cycle. Water and regular food were freely available. All steps were performed according to the guidelines of the National Institute of Laboratory Animal Health, and ethical approval was issued by the Faculty of Veterinary Medicine of Shahid Bahonar University of Kerman, Iran (IR.UK.VETMED.REC.1401.002). The animals were adapted to the laboratory conditions for a week before they were randomly divided into six groups (n = 7) as follows:

1- Control group; Twenty-four hours and just before being placed in the metabolic cage, the rats received normal saline via gavage.
2- Right nephrectomy group (RN); only subjected to right nephrectomy, without any ischemia-reperfusion.
3- Ischemia-reperfusion group (I-R); underwent right nephrectomy along with 45 minutes of ischemia in the remaining left kidney followed by reperfusion.
   Groups 2 and 3 received 1 ml of normal saline via gavage, 24 hours and just before surgery.
4- Ischemia-reperfusion + alpha-pinene groups (I-R+A) with three different doses: 2.5, 5, and 10 mg/kg Body Weight (BW) alpha-pinene. They received alpha-pinene dissolved in normal saline by gavage, 24 hours and immediately before the induction of I-R.

Induction of renal ischemia-reperfusion

To induce renal ischemia-reperfusion, the animals were first anesthetized by intraperitoneal injection of ketamine (50 mg/kg BW) and xylazine (10 mg/kg BW). The body temperature of the rats was maintained at 37°C. After laparotomy, the right nephrectomy was performed. Then, ischemia was induced by occluding the left renal pedicle for 45 minutes. The animals were then placed in a metabolic cage, and at the end of the 24 hours after reperfusion, the urine volume was

measured. Finally, they were anesthetized with CO2, and their heart blood was collected to obtain serum. In addition, one part of the left kidney was kept at -80°C to measure oxidative stress factors, and another part was stored in formalin 10% for histopathological study.

Biochemical analysis of serum and urine

Blood was centrifuged at 3,000 rpm for 20 minutes. Serum and urine creatinine and serum urea levels were measured via Biosystem Autoanalyzer (BA400, UK) using commercially available kits (KONELAB 20XT, Finland). Also, creatinine clearance (ClCr) was calculated by using the following formula: ClCr = (Ucr×V)/SCr, where UCr is urinary creatinine, V = urine volume in 24h, and SCr = serum creatinine.

Evaluation of oxidative stress in renal tissue: CAT, GPx, and MDA

1) Evaluation of catalase (CAT)

A hundred milligrams of the kidney was homogenized with one ml of phosphate buffer using a homogenizer. Then, the homogenate was centrifuged at 10,000 rpm for 10 minutes and the supernatant was separated and used as tissue extract.

Briefly, 50 mM of phosphate buffer was mixed with 15 mM of hydrogen peroxide followed by adding 100 µl of the tissue extract to the mixture, and the activity of this enzyme in the dark was measured by a spectrophotometer (Cary 50 conc, el01065009, Australia) at 240 nm[24].

2) Evaluation of Glutathione Peroxidase (GPx)

The reaction mixture consisted of 50 mM phosphate buffer with pH 7, hydrogen peroxide 0.3%, and guaiacol 0.1%. The reaction was performed by adding 20 µl of tissue extract to 2.5 ml of the reaction mixture at 25°C. Afterward, the activity of this antioxidant enzyme was performed by measuring the absorption of guaiacol due to peroxidase activity via a spectrophotometer at 470 nm[25].

3) Evaluation of Malondialdehyde (MDA)

Two-tenths of a gram of the kidney was homogenized in 5 ml of trichloroacetic acid (TCA; 0.1%), and the resulting solution was centrifuged at 10,000 rpm for 10 minutes. Next, 1 ml of the resulting supernatant was mixed with 4 ml of 20% TCA containing 0.5% thiobarbituric acid (TBA), and the mixture was incubated in a water bath at 95°C for 30 minutes. Then, it was immediately immersed in ice water, and the solution was centrifuged at 10,000 rpm for 10 minutes. The absorbance intensity was recorded at 532 nm[26].

Histological studies

The kidney tissue was fixed by using 10% formalin for 72 h. After dehydration with ethanol and clearing by xylene, the samples were embedded in paraffin. Finally, five µm thick sections were prepared and stained by the Hematoxylin-Eosin (H&E) technique.[27] Histopathological findings such as tubular occlusion, hyaline cassette formation, and necrosis were assessed by using a light microscope.

The severity of kidney damage was determined according to Jablonski *et al.*[28] A scale of 0 to 4 was used to grade the damage: zero, normal state; 1, minimal amount of damage; 2, gentle loss; 3, medium injury; and 4, severe damage. Additionally, some morphological characteristics of the kidney, including the number of glomeruli in 20 different fields, and the diameter of the glomerulus and proximal tubule were measured.

Real-time PCR (Evaluation of inflammatory factors in kidney)

The expression of inflammatory factors, such as NF-κB, TNF-α, and IL-1, and apoptotic genes Bax and Bcl-2, were evaluated by real-time PCR. Briefly, renal tissue was first homogenized with a Trizol (Zaver zist Azuma, Iran). Then, based on the chloroform-alcohol protocol, RNA was extracted from tissue samples, and the extracted RNA resulting precipitate was re-suspended in 30µl of diethylpyrocarbonate-treated water (DEPC-treated water). Finally, the concentration of the extracted RNA was measured by using a Nanodrop (A260⁄A280 ≥1.9). Following instructions, reverse transcription from the extracted RNA was performed by using a cDNA synthesis kit (Pars tous, Iran). Quantitative real-time polymerase chain reaction (qPCR) with the SYBR green (SOLIS BIODYNE) reporter dye was performed on the basis of the following protocol: a denaturation phase of 15 min at 95°C and 40 cycles for real-time PCR including 30 sec at 95°C (denaturation), 30 sec at 61°C (annealing), and 30 sec at 72°C (polymerase elongation). All steps were performed with the device Qiagene (Germany). Primer sequences and

RT-PCR segment lengths are displayed in Table 1. In qPCR, the relative mRNA levels were estimated via the expression ΔCT. To control PCR products, Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers were in separate reactions as a housekeeping gene.

Statistical analysis

Data analysis was performed by using SPSS software, version 20. Differences in mean data among experimental groups were performed by one-way analysis of variance (ANOVA) followed by the Tukey test. The results were expressed as mean ± SEM, and $P < .05$ was considered significant levels. The data relating to the histopathological changes in the experimental groups (Table 2) were analyzed by the non-parametric Kruskal-Wallis test and the Mann-Whitney test was run for pairwise comparisons.

RESULTS

The effect of alpha-pinene on kidney function

As shown in Figure 1A, a significant increase in serum urea was observed in the I-R group compared to the control and RN groups ($P < .001$). On the other hand, alpha-pinene in doses of 2.5, 5, and 10 mg/kg in I-R+A groups showed a marked decrease compared to the I-R group ($P < .001$).

In addition, about sevenfold decrease in the creatinine clearance level of the I-R group compared to control and RN groups was observed ($P < .001$) (Figure 1B). In rats receiving alpha-pinene at the dose of 2.5 mg/kg, it increased significantly ($P < .001$) compared to I-R group. However, treatment with 5 mg/kg and 10 mg/kg of this compound did not show a considerable effect on the creatinine clearance level of treated groups.

The effect of alpha-pinene on the oxidative stress factors in the kidney

Concentration of the renal MDA in the I-R group increased about tenfold compared to the control and RN groups ($P < .001$). However, MDA decreased significantly after administration of 2.5 mg/kg of alpha-pinene compared to the I-R group ($P < .001$). Noteworthy, no significant difference was observed between the I-R the I-R + A5, and A10 groups (Figure 2A).

The activity of CAT (Figure 2B) and GPx (Figure 2C) antioxidant enzymes in the I-R group showed a marked reduction compared to control and RN ($P < .01$ and $P < .001$). Its activity increased significantly from about 0.012 U/mg protein in the I-R group to about 0.024 U/mg protein in the I-R+A2.5 group ($P < .01$) (Figure 2B). Also, the activity of glutathione peroxidase in the I-R+A2.5

Table 1. Details of the primers utilized in the study.

Primer name	Primer sequence	PCR product size	NCBI accession number
GAPDH	F: GTCTTCACCACCACGGAGAAGGC R: ATGCCAGTGAGCTTCCCGTTCAGC	392	NM_017008.4
TNF-α	F: ACCAGCAGATGGGCTGTACCTTAT R: ATGAAATGGCAAATCGGCTGACGG	107	NM_012675.3
NF-κB	F: AGAGCAACCGAAACAGAGAGG R: ATATGCCGTCCTCACAGTGC	227	NM_001276711.1
IL-1β	F: AAGACACGGGTTCCATGGTGAAGT R: TGGTACATCAGCACCTCTCAAGCA	98	NM_031512.2
BAX	F: CCCGAGAGGTCTTCTTCCGTG R: CCAGCCCATGATGGTTCTGAT	155	XM-039087751.1
BCL-2	F: CATGTGTGTGGAGAGCGTCAA R: GCCGGTTCAGGTACTCAGTCA	88	NM_016993.2

Table 2. The effect of alpha-pinene administration on histopathological changes caused by I-R injury in experimental groups.

Groups	Congestion	Necrosis	Inflammation	Casts	Atrophy	Tubular vasodilation
Control	0	0	0	0	0	0
RN	0	0	0	0	0	0
I-R	4 **	4**	4**	4 **	4 **	3 **
I-R + A2.5	2&&	2&&	1.5&&	2&	2&	1.5&&
I-R + A5	3.5	3.5	3.5	3.5	3.5	3
I-R + A10	3.5	3.5	3.5	3.5	3.5	3

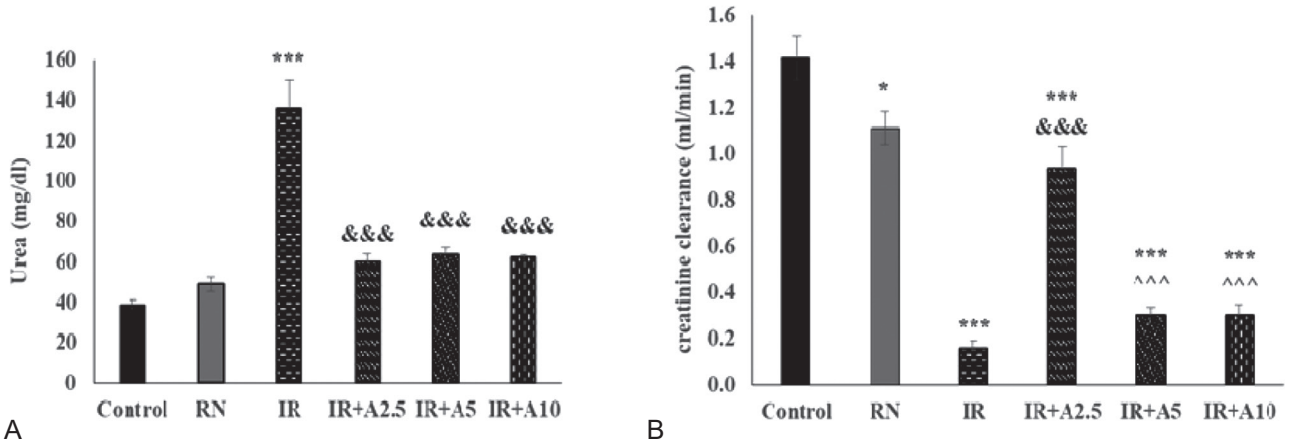

Figure 1. Effect of alpha-pinene on serum Urea (A) and creatinine clearance (B) levels in the experimental groups (mean ± SEM). RN (Right nephrectomy), I-R (Ischemia-reperfusion), I-R + A2.5, A5, and A10 (Ischemia-reperfusion + 2.5, 5, 10 mg/kg of alpha-pinene). ***P < .001 vs control and RN groups, *P < .05 vs control; &&& P < .001 vs I-R group; ^^^P < .001 vs IR + A2.5 group.

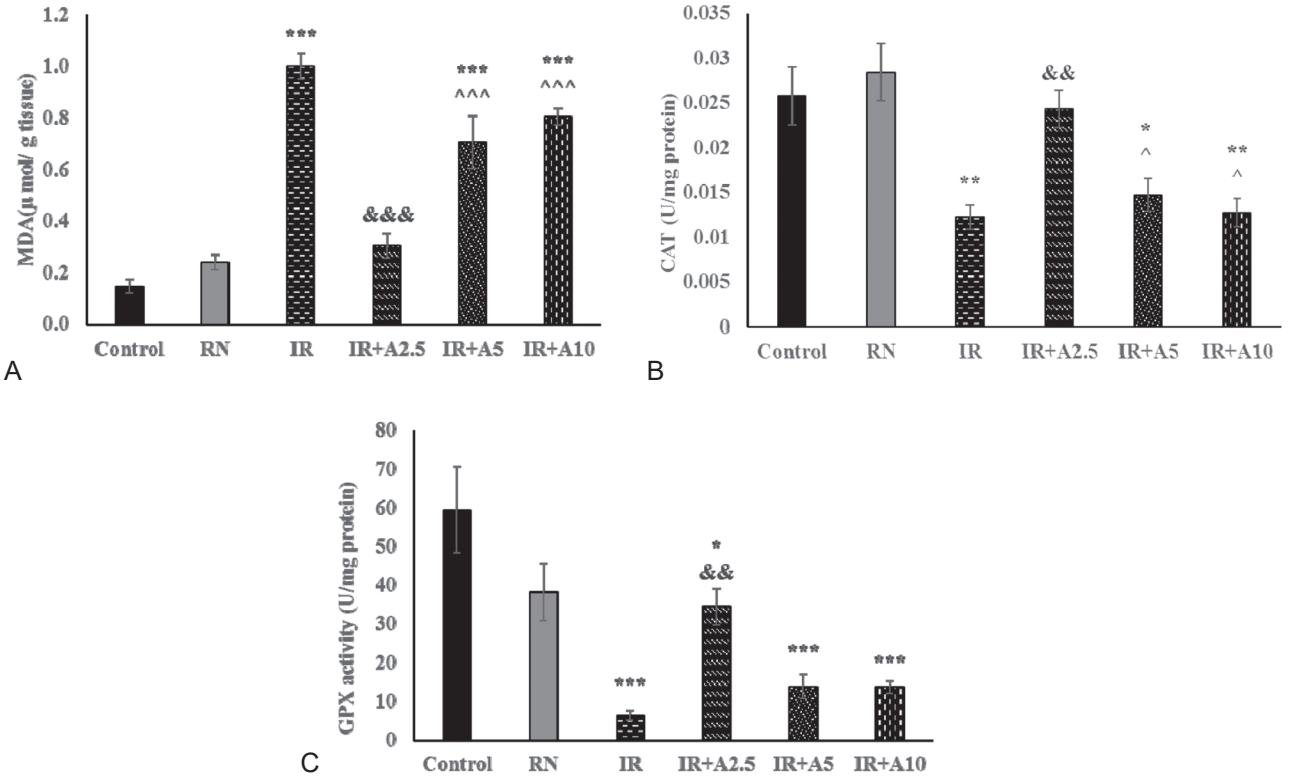

Figure 2. Effect of alpha-pinene on oxidative stress factors in the kidney: (A) MDA, (B) CAT, and (C) GPx in the experimental groups (mean ± SEM). RN (Right nephrectomy), I-R (Ischemia-reperfusion), I-R + A2.5, A5, and A10 (Ischemia-reperfusion + 2.5, 5, 10 mg/kg of alpha-pinene). ***P < .001, **P < .01, *P < .05 vs control and RN groups; &&& P < .001, && P < .01 vs I-R group; ^^^ P < .001,^ P < .05 vs I-R + A2.5 group.

group showed a significant increase compared to the I-R group (P < .01).

Histopathological changes in kidney tissue of experimental groups

The results of the histopathology examination are shown in Figure 3A-F, Figure 4, and Table 2. In the control and RN groups, normal structure of the kidneys including glomeruli and tubules was observed (Figure 3A, B). In I-R group, severe pathological lesions such as tubular dilatation, glomerular atrophy, tubular hyaline cast formation,

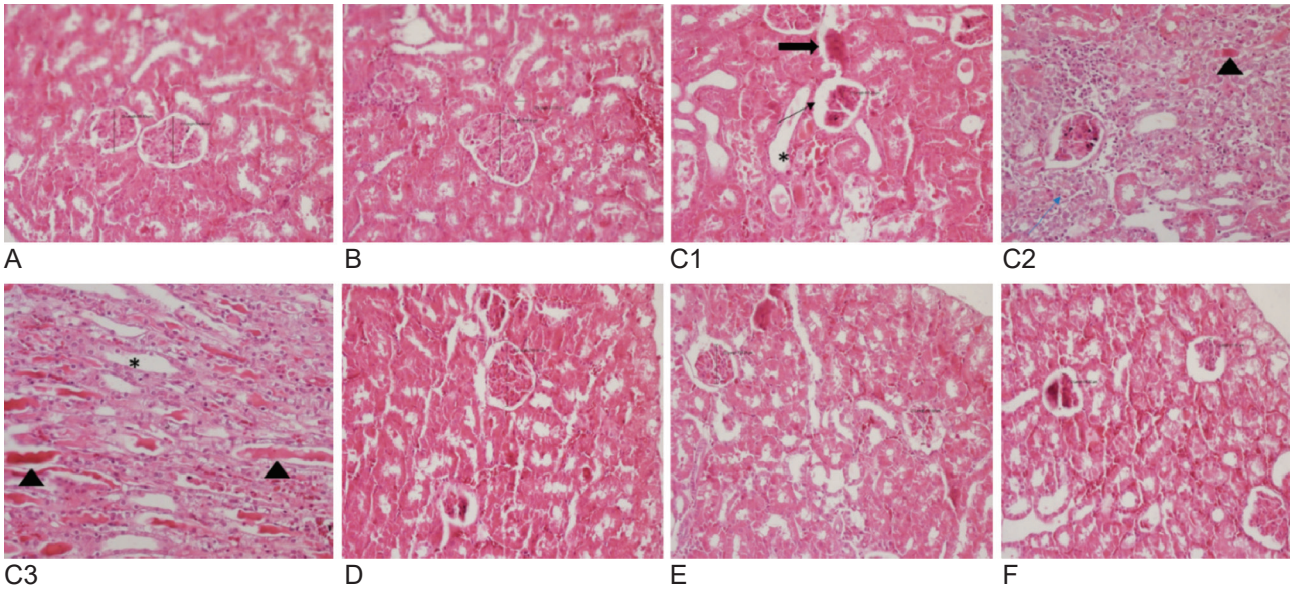

Figure 3. Kidney histopathology investigation with H&E staining in experimental groups. (A, B): Normal kidney structure in control and nephrectomy groups; (C1-C3): changes caused by I-R induction in the untreated group: indicating glomerular atrophy, and congestion (black arrow), cast formation (black arrowhead), inflammation (blue arrow), tubular vasodilation (asterisk); D-F: animals underwent I-R+A and restoration of some degrees of kidney damage in the group treated with a dose of 2.5 mg/kg alpha-pinene (D).

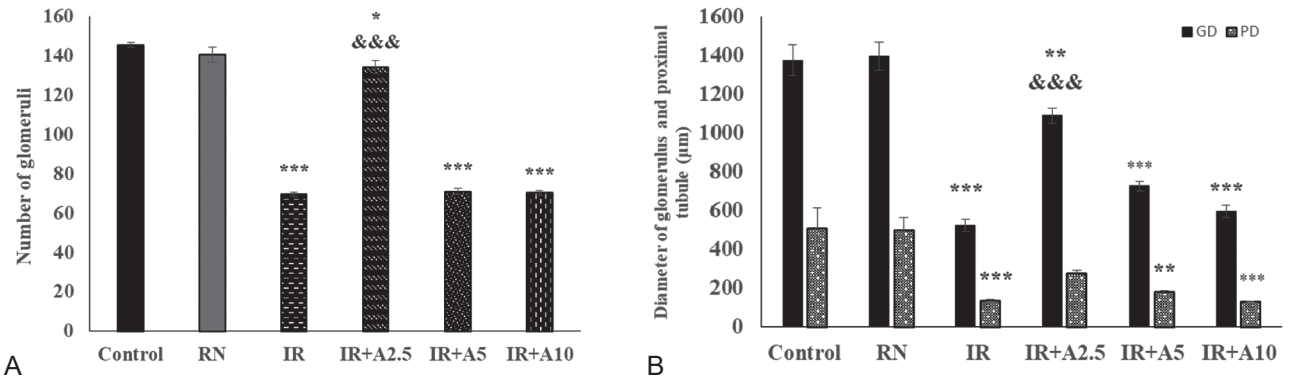

Figure 4. Evaluation of kidney morphology in the studied groups (mean ± SEM). (A) The number of glomeruli in 20 different fields, (B) the Diameter of the glomerulus and proximal tubule. RN (Right nephrectomy), I-R (Ischemia-reperfusion), I-R + A2.5, A5, and A10 (Ischemia-reperfusion + 2.5, 5, 10 mg/kg of alpha-pinene), GD (Glomerular diameter), PD (Proximal tubule diameter). ***P < .001, **P < .01 vs control and RN groups; &&& P < .001 vs I-R group.

congestion, inflammation, and acute tubular necrosis were observed (P < .01) (Figure 3C1-C3) (Table 2). In contrast, administration of alpha-pinene (2.5 mg/kg) showed a significant improvement (P < .01) in all cases of pathological damage compared to the I-R group (Figure 3D) (Table 2,). Although the treatment with alpha-pinene (2.5 mg/kg) aimed to mitigate damage, higher doses of alpha-pinene (5 and 10 mg/kg) did not exhibit a significant difference compared to the I-R group (Figure 3E, F) (Table 2). Also, as shown in Figure 4, a significant decrease in the number (A) and diameter of the glomerulus and the diameter of the proximal tubule (B) is observed in the I-R group compared to the Control and RN groups (P < .001).

Finally, there was a significant improvement in the morphological changes in the I-R + A2.5 group (P < .001) (Figure 4, A & B).

The effect of alpha-pinene on the expression of Bax and Bcl-2

Induction of renal I-R increased the Bax expression (Figure 5A) while decreasing Bcl-2 expression (P < .001) (Figure 5B). Nevertheless, the treatment of rats with alpha-pinene (2.5 mg/kg) caused a marked reduction in mRNA expression

of Bax ($P < .001$) and an increase in expression of Bcl-2 ($P < .001$) (Figure 5B).

The effects of alpha-pinene on TNF-α, NF-κB, and IL-1β

Expression of TNF-α in the I-R group increased significantly compared with the control and RN groups (Figure. 6A) ($P < .001$). Besides, alpha-pinene significantly reduced TNF-α compared to the I-R group ($P < .001$).

I-R group showed an increased expression of NF-κB in comparison to the control and RN groups ($P < .001$) (Figure. 6B). Further, in the group treated with the effective dose of alpha-pinene (2.5 mg/kg), the expression of this factor showed a significant decrease compared to the I-R group ($P < .001$). The rate of IL-1β mRNA increased in the I-R group compared to the Control and RN groups ($P < .001$)

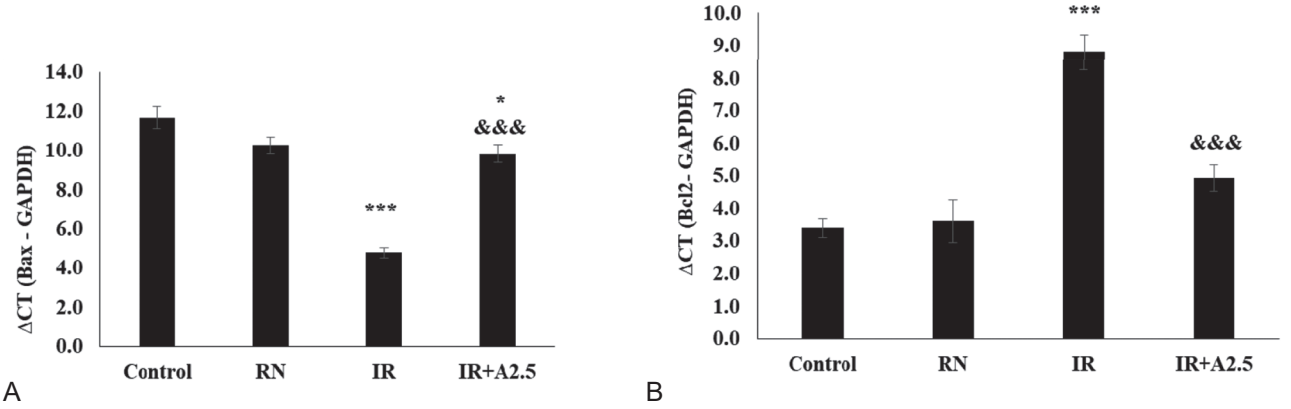

Figure 5. Investigation of Bax (A) and Bcl-2 (B) mRNA expression in the different studied groups (mean ± SEM). RN (Right nephrectomy), I-R (Ischemia-reperfusion), I-R + A2.5 (Ischemia-reperfusion + 2.5mg/kg of alpha-pinene). ***$P < .001$, *$P < .05$ vs control and RN groups; &&& $P < .001$ vs I-R group.

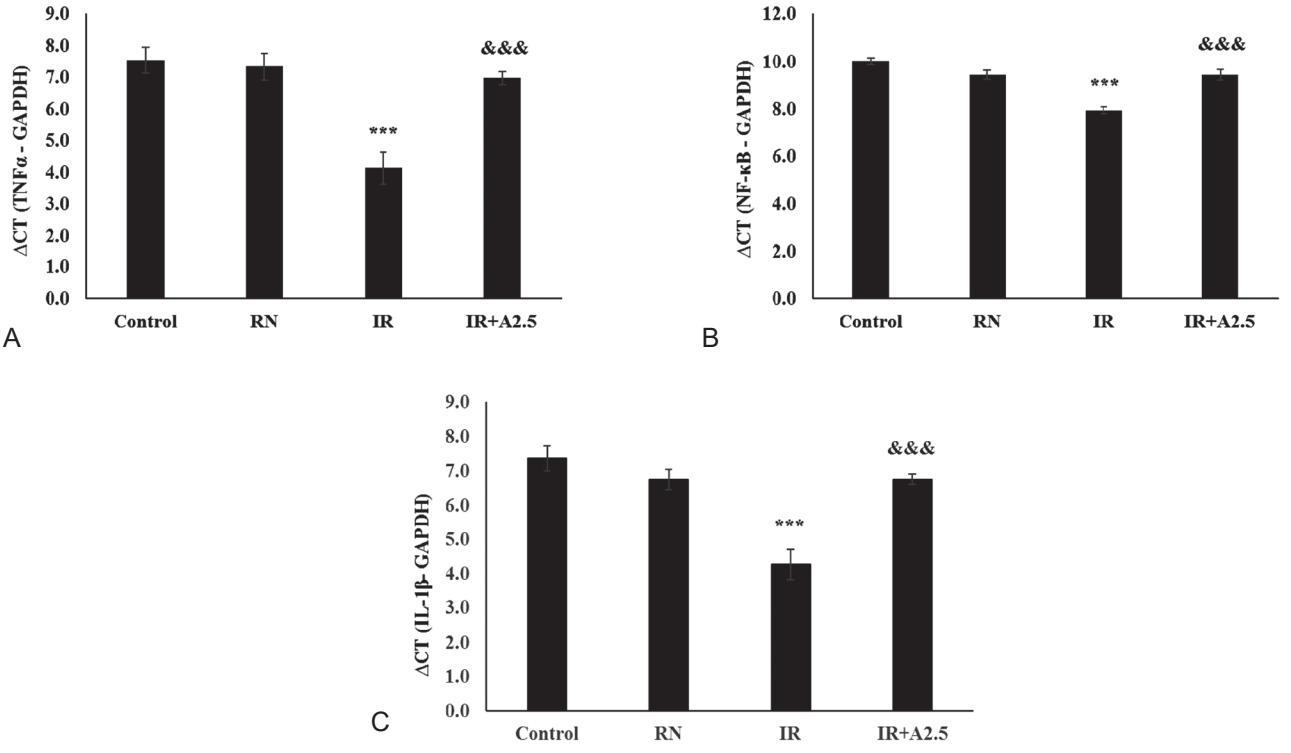

Figure 6. The effects of pretreatment with alpha-pinene on pro-inflammatory cytokines mRNA expression such as TNF-α (A), NF-ƙB (B), and IL-1β (C) in renal tissue of the various groups studied (mean ± SEM). RN (Right nephrectomy), I-R (Ischemia-reperfusion), I-R + A2.5 (Ischemia-reperfusion + 2.5mg/kg of alpha-pinene). ***$P < .001$ vs control and RN groups; &&& $P < .001$ vs I-R group.

(Figure 6C). On the other hand, the expression of IL-1β was remarkably downregulated in I-R+A2.5 compared to the I-R group ($P < .001$).

DISCUSSION

Renal ischemia-reperfusion (I-R) injury is the leading cause of renal dysfunction following conditions such as organ transplantation and vascular surgery.[29] Tissue damage due to the return of blood flow to ischemic tissue is considered reperfusion ischemic injury.[30] The sudden increase in oxygen uptake following the re-establishment of blood flow in ischemic tissue leads to the overproduction of free radicals which in turn causes damage to cellular proteins, lipids, DNA, and ultimately cell apoptosis and necrosis.[29] In this study, the impact of oral administration of alpha-pinene on the recovery process of renal injury induced by I-R in male rats was investigated.

Our results illustrated that renal I-R causes impaired renal function, indicated by increased serum urea, and decreased creatinine clearance. Acute kidney damage occurs primarily due to reduced blood flow to the kidney which leads to decreased oxygen delivery to the tissue.[5] These conditions reduce glomerular filtration rate and urine output, affecting blood urea nitrogen and serum creatinine.[31,32] Alpha-pinene, via targeting oxidative stress, inflammatory markers, and apoptosis pathways has a powerful effect on improving kidney function.

During renal I-R, decreased renal blood flow followed by its return, increases ROS production, which stimulates the onset of pathological cascades such as increased oxidative stress, the release of inflammatory cytokines, and tubular cell injury.[33] The levels of antioxidant enzymes such as GPx, SOD, and CAT decrease with increasing ROS production. In contrast, damage to cell membranes due to free radicals increases lipid peroxidation, leading to an increase in the levels of MDA.[34,35] The current study also showed that I-R significantly reduced the activity of antioxidant enzymes CAT and GPx and increased the amount of MDA. However, alpha-pinene reduced MDA and increased antioxidant activity. Previous studies on alpha-pinene have reported the antioxidant effects of this compound, including the reduction of lipid peroxidation and increasing the protein expression of antioxidant enzymes such as GPx, CAT, and SOD in ischemic stroke.[36]

In several studies, the inflammatory response has been known as an essential mechanism inducing renal injury after I-R.[37,38] In our research, like another study,[9] I-R resulted in increased mRNA expression of some inflammatory cytokines such as TNF-α, NF-κB, and IL-1β.

In addition, the effective dose of alpha-pinene (2.5 mg/kg) significantly attenuates the expression of the inflammatory cytokines, possibly by reducing the penetration of these factors in the kidney of I-R-treated rats. Hypoxia, due to decreased renal blood flow, raises the production of inflammatory factors. This, in turn, increases the infiltration of inflammatory factors into the tissue and intensifies kidney cell damage. As a result, it causes direct damage to DNA or the induction of apoptosis.[39] Increased TNF-α expression was associated with cell death via activation of caspase-induced apoptosis pathways.[40] Also, the production and secretion of pro-inflammatory cytokines in the affected areas can stimulate and release other types of inflammatory mediators.[41] Under conditions of oxidative stress, the expression of genes involved in inflammation, including NF-κB, is affected, which plays a vital role in regulating processes such as apoptosis and inflammation. This factor also stimulates the expression of pro-inflammatory cytokines such as IL-1β and TNF-α, facilitating the penetration of inflammation cells.[42] Some studies have shown that intraperitoneal injection of alpha-pinene has a neuroprotective effect against cerebral ischemic-reperfusion injury.[43] This compound prevents the increase in expression IL-1β and TNF-a in the brain.[44] It has also been showed that TNF-α, IL-1β, IL-6, and NF-κB production is reduced during cerulein-induced acute pancreatitis with alpha-pinene pretreatment.[18] Furthermore, the anti-inflammatory activity of alpha-pinene in peritoneal macrophages of rats has been demonstrated by lowering inflammatory mediators including IL-6, TNF-α, and COX-2, and mainly by inhibiting MAP / NF-κB pathways.[45]

In our study, the expression of genes involved in apoptosis was also studied as one of the main mechanisms of renal tubular cell death during renal I-R injury, similar to another study.[46] Apoptosis contributes to cell death in renal I-R by altering Bax and Bcl-2 expression.[47,48] Consequently, these conditions have shown that I-R increases Bax/Bcl-2

proportion and thus promotes apoptosis in humans, mice, and rats kidneys. Wei *et al.* demonstrated the importance of apoptosis-promoting genes by knocking out Bax and Bak in mice with I-R-induced acute renal failure. Their study showed that deletion of Bax or Bak genes in the proximal tubular cells protects against I-R damage in experimental groups.[49] Administration of alpha-pinene to rats significantly reduced and increased the expression of Bax and Bcl-2 apoptotic proteins, respectively, compared with the I-R group. These results are consistent with the results obtained by the studies of Karthikeyan and Khoshnazar, which showed that alpha-pinene reduces the rate of apoptosis by maintaining the integrity of the mitochondrial membrane, restoring Bacl-2, and suppressing Bax gene expression that altogether prevent the activation of caspase-9 and 3.[50,51] It was also reported that alpha-pinene can inhibit the apoptotic pathway by decreasing c-Jun N-terminal kinase (JNK) activity.[52] These findings showed that the ameliorating effects of alpha-pinene on I-R damage may be performed to some extent by suppressing the expression of the apoptosis-promoting gene (Bax) along with the induction of anti-apoptotic gene expression (Bcl-2).

In the histopathological examination of the kidney of the I-R group, changes such as proximal tubular necrosis and atrophy were observed, and these results were similar to previous studies.[40,53] Also, the results of this study showed that the tissue damage was relatively improved in rats treated with alpha-pinene (2.5 mg/kg).

Despite recent advancements in the treatment of renal failure, of which I-R injury is the most common cause, it remains a major problem, and up to now, there is no definitive cure for it, so the likely underlying mechanisms involved, need further investigation.[40,54]

CONCLUSION

The results of the present study showed that the induction of renal ischemia-reperfusion in the animal model causes renal dysfunction, histopathological changes, increased lipid peroxidation, the release of inflammatory factors, and apoptosis in the kidney. On the contrary, due to the anti-inflammatory and antioxidant effects of alpha-pinene, oral pretreatment of rats with this compound led to improved renal function, reduced expression of inflammatory factors, and also regulated expression of apoptotic proteins in the kidney.

AUTHOR CONTRIBUTIONS

Design of the study: Ali Gol. Performing the experiments: Sedighe Khodsooz. Analyzing the data: Ali Gol and Sedighe Khodsooz. Pathology Assessment of the Kidney: Shahriar Dabiri. Writing the manuscript: Sedighe Khodsooz. Reviewing the manuscript: Ali Gol.

AVAILABILITY OF DATA

The data used and analyzed statistically in this study are available upon reasonable request from the corresponding author.

DISCLOSURE STATEMENT

There is no relevant financial or non-financial competing interests to report.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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