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Original Research

Protective effect of gel form of gastric gavage applicated aloe vera on ischemia reperfusion injury in renal and lung tissue

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Abstract: The aloe vera plant has become increasingly popular in recent years. This study aimed to research the effect of aloe vera to prevent renal and lung tissue damage in an experimental ischemia–reperfusion (I/R) injury model. The study included 21 male Wistar Albino rats, which were categorized into control group, n = 7 (no procedures), Sham group n = 7 (I/R); and aloe vera therapy group, n = 7 (aloe vera and I/R). Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA) were evaluated from lung and kidney tissues for biochemical investigations. As histopathological, hematoxylin and eosin and anti-iNOS were also examined. In biochemical investigations, SOD, CAT, and GPx levels of the Sham group were found to be lower compared with the other groups ($P < 0.05$). The aloe vera therapy group was not statistically different from control groups but significantly different compared with the Sham group. In the same way, the MDA levels of kidney and lung tissues were statistically significant in the aloe vera therapy group, compared to the Sham group. In the Sham group, the peribronchial and perialveolar edema were observed in lung parenchyma. Also, excess interstitial hemorrhage, leukocyte infiltration, and alveolar wall thickening were identified in ischemic groups. The histopathological changes were much lighter than in the aloe vera therapy group. In renal tissues, excess epithelial cell deterioration, tubular desqumination, and glomerular atrophy were observed in the Sham group. The histopathological changes were markedly reduced in the aloe vera therapy group. In the kidney and lung tissue, the level of iNOS activity in the Sham group was significantly higher than in the control and aloe vera therapy group. This study indicated that aloe vera is protective against oxidative damage formed by I/R in distant organs like the lungs and kidneys.

Key words: Aloe vera; I/R; Ischemia and reperfusion; Kidney; Lung.

Introduction

Ischemia is the development of reversible or irreversible cell/tissue damage, linked to insufficient blood flow permeating organs or tissues (1). After ischemia, there are many metabolic and structural changes in cells. Ischemia disrupts oxidative phosphorylation in the cell, causing reductions in intracellular adenosine triphosphate (ATP) and phosphocreatine synthesis (2). This situation disrupts the ionic pump function linked to ATP in the cell membrane, causing more calcium, sodium, and water to enter the cell (3). During ischemia, the destruction of adenine nucleotide increases. This increases the accumulation of reactive oxygen species (ROS) precursor hypoxanthine within the cell. After ischemia, when blood flow is restored to the region again (reperfusion) and intracellular molecular oxygen is provided again, ROS rapidly forms (4). To prevent irreversible cell damage, reperfusion to the organ/tissue is essential. However, reperfusion may cause more damage to the ischemic tissue than ischemia occuring in the tissue/organ.

For a majority of time, the ischemia and reperfusion damage does not remain limited to that organ. With many intervening active systems and toxic mediators, it may affect a variety of organs such as the lungs, kidneys, liver, and heart and cells in every organ like endothelium and epithelium (5). Antioxidants have been used to prevent this damage with the help of antioxidant enzymes. Malondialdehyde (MDA) is the final product of lipid peroxidation. It is frequently used to define oxidative stress; superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) represent antioxidant enzymes (6).

The study assessed the effect of numerous plants on kidneys and lungs against ischemia–reperfusion (I/R) deterioration (7, 8). Aloe vera has been increasing in popularity in recent years. In the medical field, aloe vera has displayed anti-inflammatory, immunomodulator, antitumor, anti-aging, and positive effects on wound healing (9). Studies also reveal the cardio-protective effect of aloe vera (10) and its prevention of nerve damage caused by I/R (11).

This study aimed to research the effect of aloe vera to prevent renal and lung tissue damage in an experimental I/R injury model.

Materials and Methods

Ethical issues

After obtaining permission from Çanakkale Onsekiz Mart University (ÇOMÜ) Local Ethics Committee (2014/12-08), our study was completed in the Experimental Animals Application and Research Center.

Animals

The study included 21 male Wistar Albino rats weighing 250–300 mg, aged 8–12 weeks. The rats were obtained from the ÇOMÜ Experimental Animals Application and Research Center. Rats adapted to the environment were used in this study. They were randomly chosen and divided into three groups of seven. During the study, the subjects were kept in the ÇOMÜ Experimental Research Center in special cages and given appropriate nutrition. The rats were allowed free access to food and water at a controlled temperature (23–25°C) and lighting environment (8:00– 20:00 light, 20:00–08:00 dark). All surgical and experimental procedures were in accordance with the animal care guidelines of the ÇOMÜ Experimental Research Center. Aloe vera in gel form used in the research was purchased from Herbalife Turkey distributor (Herbalife Inc., İstanbul, Turkey).

Group 1: Control group n = 7 (no medication or surgical procedures).

Group 2: Sham group n = 7 (45 min abdominal aorta ischemia, 24 h reperfusion before sacrifice).

Group 3: Aloe vera therapy group n = 7 [1 month of daily aloe vera application (30 mg/kg) with gastric gavage, 45 min abdominal aorta ischemia, 24 h reperfusion before sacrifice].

Surgical procedure

All groups were given anesthesia with 5 mg/kg xylazine (Bayer, İstanbul, Turkey) and 50 mg/kg ketamine hydrochloride (Parke Davis, İstanbul, Turkey). Procedures were completed with spontaneous respiration and room air. The rats were entered with a midline dermal-subdermal incision in the supine position under sterile conditions on the operating table. A surgical procedure for peripheral nerve damage was described by Schmelzer in 1989 (12). The intestines were turned to the right and the abdominal aorta was reached through the midline. In groups with ischemia induced, the abdominal aorta and vena cava inferior were carefully dissected and separated. The abdominal aorta was clamped and turned immediately below the renal artery and above the bifurcation, and after 45 min clamping the clamps were removed and reperfusion was provided. The abdomen was closed appropriately. All groups were sacrificed with high dose anesthetic material after the 24th hour. The renal and lung tissues were completely removed. Half of the removed tissue was stored in formalin. The tissues were sent to the laboratory for histopathological investigation and tissue biochemistry for MDA, CAT, SOD, and glutathione peroxidase (GPx) activity levels.

Biochemical analyses

Immediately after tissues were removed, they were stored at -80°C. For biochemical analyses, tissues were homogenized in appropriate buffer media separately prepared for each method and supernatants were removed. The SOD, CAT, GPx activities, and MDA levels were measured with extremely sensitive enzyme-linked immunosorbent assay (ELISA) spectrophotometry. The studies were repeated twice. Total protein concentrations were completed in accordance with the Bradford method (Sigma Aldrich, Bradford reagent-B6916-1KT, USA). All results were determined as mean ± standard deviations per mg protein (SD/mg protein).

SOD activity

IC50 (SOD activity 50% inhibition) values were determined colorimetrically using an SOD assay kit (Biovision-K335-100; Milpitas, CA95035, USA) at 450 nm. Results are given as U/mL per mg protein [U/(mL . mg protein)].

CAT activity

Determined using STA-341, Cell Biolabs' Oxiselect Catalase Activity Assay Kit, Colorimetric. Results obtained by ELISA spectrophotometry are stated as U/(mL . mg protein).

GPx activity

Determined with ELISA spectrophotometry using Biovision-K762-100; Milpitas, CA95035, USA, Glutathione Peroxidase Activity Colorimetric Assay Kit. Results are given as mU/mL per mg protein [mU/(mL . mg protein)].

Tissue MDA levels

Determined using Biovision-K739-100; Milpitas, CA95035, USA, Lipid Peroxidation (MDA) Colorimetric/Fluorometric Assay Kit. Results are stated as nmol per mg tissue (nmol/mg tissue).

Histopathological examination

Kidney and lung tissues were removed from all animals of groups, fixed in 10% formaldehyde solution at room temperature for 48 h. The tissue samples were dehydrated and embedded in paraffin according to standard histological procedures. Paraffin blocks were cut into 5 µm with a microtome (SLEE, cut5062) for general pathological examination and immunohistochemical analysis. Slides were stained with hematoxylin and eosin. Histological preparations were examined under a light microscopy Olympus CX41 (Olympus, Japan) using different magnifications to evaluate the degree of lung and kidney injury. Histopathological changes in lung tissue; cell infiltration, lung edema, congestion and intra-alveolar hemorrhage were evaluated. These findings were graded as follows: 0, normal histological architecture; 1, mild; 2, moderate; and 3, severe changes in lung tissue. The lung injury score possible was 12 (13). Renal injury was evaluated and classified as follows: 0, normal histological architecture; 1, tubular cell swelling, brush border loss and nuclear condensation, with up to one third of the tubular profile showing nuclear loss; 2, as grade 1, but greater than one third and less than two thirds of tubular profile showing nuclear loss; and 3, greater than two thirds of tubular profile showing nuclear loss (14). A minimum 100 intersections for each animal were examined by 2 separate investigators with

Table 1. Superokside dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) activities and levels of malondialdehyde (MDA) of kidney and lung tissues.

Groups	SOD (U/ml.mg protein)	CAT (U/ml.mg protein)	GPx (mU/ml.mg protein)	MDA (nmol/mg tissue)
Kidney				
Control	54.20±7.17	33.47±3.80	41.44±6.54	9.71±3.59
Abd-I/R	12.26±5.18	7.43±1.72	7.49±2.47	32.45±4.67
Abd- I/R+AV	49.31±7.98[β]	27.40±3.65 [β]	39.83±3.94 [β]	12.58±3.81 [β]
Lung				
Control	45.00±4.70	36.52±5.98	31.40±2.67	7.51±1.10
Abd-I/R	26.17±4.79	4.48±1.60	11.90±2.18	17.02±1.61
Abd- I/R+AV	38.92±3.88[β]	26.16±4.29[β]	23.24±2.31[β]	9.46±2.11[β]

Note: AV: AloeVera, Abd-I/R: Abdominal Ischemia Reperfusion. Means by the letter [β] is significantly different to the One-way ANOVA-Tukey's test in each coloumn ($p < 0.05$). The results were defined as the mean ± standard deviation (SD).

light microscopy.

Immunohistochemical examination of iNOS

The tissue sections were deparaffinized in xylene and rehydrated in descending concentrations of ethanol, followed by antigen retrieval in sodium citrate buffer (10mM, pH 6) for 10 min. Endogenous peroxidase was inhibited by incubation with 3% hydrogen peroxide for 10 min. at room temperature. The nonspecific binding of antibodies was blocked by incubation with a blocking serum (Ultra V Block, LabVision) at room temperature for five min. The primary antibody, Anti-iNOS (ab3523) was incubated at 4°C overnight. After incubation with the primary antibody, the tissue sections were washed with phosphate-buffered saline and incubated with the biotinylated secondary antibody (Ultra Vision Detection System-HRP kit, Thermo Scientific/Lab Vision). Streptavidin peroxidase (Ultra Vision Detection System-HRP kit, Thermo Scientific/Lab Vision) was then added at room temperature for 10 min. The chromogen 3-amino-9-ethyl-carbazole (AEC Substrate System, Thermo Scientific/Lab Vision) was used and the sections were counterstained with hematoxylin. Anti-iNos immunoreactivity was examined under light microscopy and measured using the Image J software.

Statistical analysis

For biochemical investigations, results were subjected to one-way analysis of variance using SPSS 21.0 software (SPSS Inc., USA). Differences among the groups were obtained using Tukey's test option. Statistical significance was accepted as $P < 0.05$. All data were expressed as mean ± SD in each group. For histopathological examinations, results were reported as the mean ± SD. While the nonparametric Kruskal–Wallis test was used to compare the groups, the Mann–Whitney *U* test was used for binary comparisons. The Spearman correlation test was used to evaluate the relationship between the variables, and $P < 0.05$ was considered statistically significant.

Results

Biochemical investigations

The mean and SD values of SOD, CAT, GPx, and MDA in each group are given in Table 1. The SOD, CAT, and GPX levels of abdominal I/R group were lower compared with the other groups and this was statistically significant ($P < 0.05$). The aloe vera therapy group was not statistically different from control groups ($P > 0.05$) but significantly different compared with the I/R group. In the same way, the MDA levels of kidney and lung tissues were statistically significant ($P < 0.05$) in the aloe vera therapy group, compared with the abdominal I/R group.

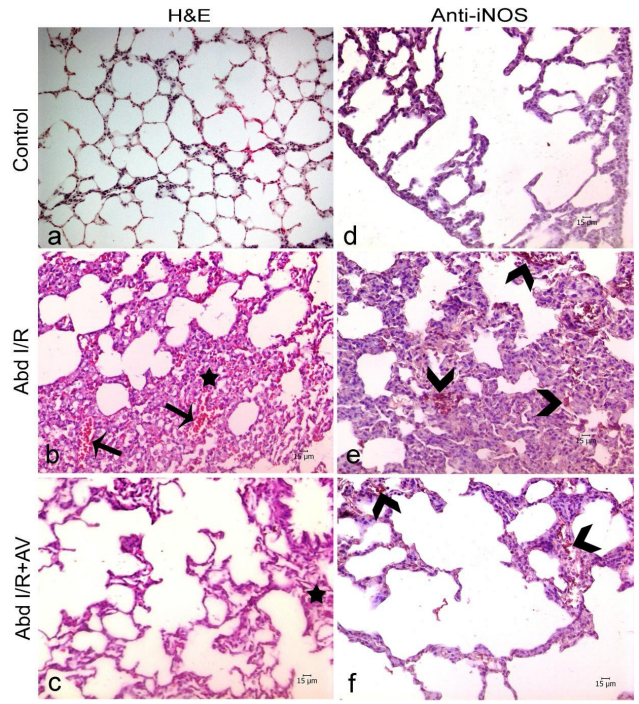

Figure 1. Hematoxylin-eosin (H&E) and Anti-iNOS staining of lung tissue (a – c; H&E, d – f; iNOS). a) Normal lung tissue b) Abd I/R group demostrates massive alveolar, perivascular and interstitial edema c) Abd IR+AV reveals minimal edema and infiltration (thin arrow; hemorrhage, asterisk; perivascular or interstisial edema, arrowhead; iNOS positive cells, Magnification; 200X).

Histopathological findings

The control group showed normal lung architecture; inflammatory cell infiltration and hemorrhage were nearly normal (Fig. 1a). In the Abd-I/R, peribronchial and perialveolar edema were observed in the lung parenchyma. Also, excess interstitial hemorrhage, leukocyte infiltration, and alveolar wall thickening were identified in ischemic groups (Fig. 1b). Histopathological changes were much lighter than in Abd-I/R + aloe vera therapy group when compared to the Abd-I/R group (Fig. 1c). Lung injury scores are summarized in Table 2. The

Table 2. Histopathological injury scores of the lung and kidney tissues.

	Lung	Kidney
Control	0	0
Abd I/R	9.6 ±1.2[a]	2.6±0.5[a]
Abd IR+AV	4.5±1.7[a,b]	1.7±0.6[a,b]

a; p<0.05 vs control group, b; p<0.05 vs Abd-I/R group.

control group was observed with respect to normal histological renal architecture (Fig. 2a). Excess epithelial cell deterioration, tubular desqumination, and glomerular atrophy were observed in Abd-I/R group (Fig. 2b). Histopathological changes were markedly reduced in the aloe vera therapy group (Fig. 2c). Total lung injury scores are shown in Table 2.

Immunohistochemical findings

The iNOS expression in lung tissue is depicted (Figure 1). iNOS activity was particularly observed in proximal and distal tubular epithelial cells in the renal tissue. Type II pneumocytes and inflammatory cells showed iNOS positive reaction in lung tissue examinations. In the kidney and lung tissue, the level of iNOS activity in the Abd-I/R group was significantly higher than in the control and aloe vera therapy group ($P < 0.05$, Table 3). iNOS immunoreactivity was observed only in tubular cells in the kidney tissue (Figure 2).

Discussion

I/R injury does not remain limited to the organ it affects the majority of the time, but affects a variety of organs including the lungs, kidneys, liver, and heart (5). Clinically, some complications linked to distant organ failure after reperfusion injury may even cause death. Reperfusion injury to lungs and kidneys frequently occurs after cardiopulmonary bypass, pulmonary thromboendarterectomy, and lung transplantation. During ischemia and reperfusion afterward, mediators released into circulation may cause macrophage activation in nearly all organs and produce inflammatory cytokines (like tumor necrosis factor α) that can activate vascular endothelium (15, 16). ROS and inflammatory leukocytes resulting from I/R injury form a significant mechanism for distant organ injury (17). Superoxide radicals are formed by the addition of an electron to an oxygen molecule. They are transformed into non-toxic products by antioxidant enzymes of SOD, CAT, and GPx (18).

Aloe vera is a plant that has been intensely studied in recent years in the medical field. It has positive effects on wound healing (19). Some glycoproteins in gel forms of aloe vera are reported to show antitumor and antiulcer efficacy in dermal tissues (20). Many in vivo and in vitro studies have shown the anti-inflammatory

Table 3. Relative density for iNOS activity of lung and kidney tissues.

	Lung	Kidney
Control	2.6±1	2.5±1.1
Abd-I/R	11±2.4[a]	35±11[a]
Abd-IR+AV	5.6±2.4[a,b]	18±4.2[a,b]

a; p<0.05 vs control group, b;p<0.05 vs Abd-I/R group.

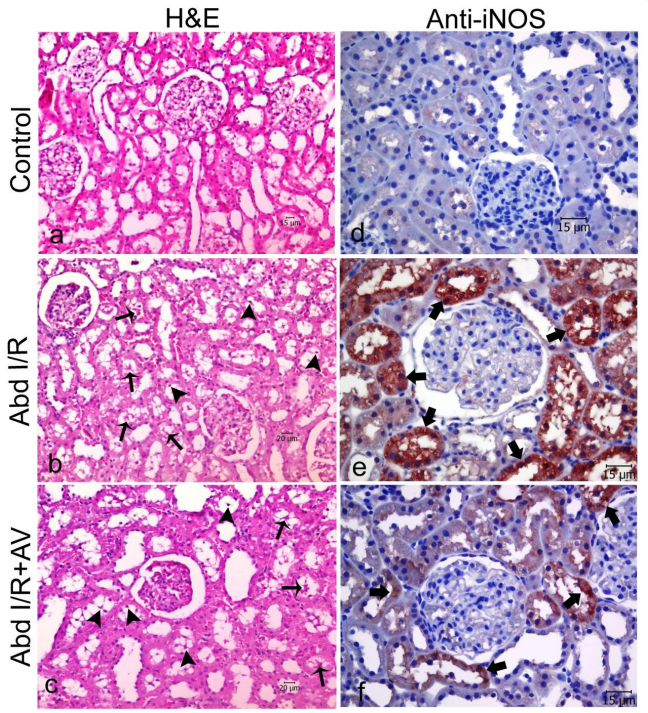

Figure 2. Figure illustrates H&E and Anti-iNOS examination of renal tissue (a – c; H&E, d– f; iNOS). a) Regular glomerular and tubular architecture in control group b) Tubular desquamination and tubular necrosis observed in Abd I/R group c) Decreased histopathological changes detected in Abd I/R+AV group (thin arrow; tubular desquamination, arrow head; degenerative cells, thick arrow; iNOS positive tubular cells, Magnification; H&E; 200X, Anti-iNOS;400X).

efficacy of aloe vera through bradykinase activity (21). Immune modulator efficacy is reported (22). The anti-aging effects of aloe vera are shown, with the reduction of acne, wrinkles, and erythema (23). There are studies on its antibacterial, antiviral, and antifungal efficacy (24-26).

MDA is one of the most important products of membrane lipid peroxidation, and its presence in tissue reveals tissue injury (27). In this study, while clear increases were observed in lung and renal tissue MDA levels in the I/R (Sham) group, no significant increase was observed in the aloe vera therapy group. The histopathological investigation showed similar results. While clear damage was observed in both the renal tissue and lung tissue in the control group, minimal edema and infiltration were identified in the tissue in the aloe vera therapy group. Significant falls were observed in SOD, GPx, and CAT values in the kidney and lungs of animals in the Sham group, while minimal changes were observed in the animals given aloe vera. These results may be considered to show the protective effect of aloe vera on tissue damage caused by oxidative stress in distant organs during I/R. To the best of our knowledge, in the existing literature there is no study showing the protective effect of aloe vera against injury in distant tissues from I/R. Additionally, an antioxidant effect of aloe vera is observed. In a study by Benson et al. (28) an extract obtained from aloe vera was protective against free radical injury and at the cellular level showed antioxidant properties. Saada et al. (29) in a study of rats fed with aloe vera reported it was protective against liver, lung, and kidney injury due to radiation exposure. Their

study, in parallel to the present study, found significant recovery in SOD, GPx, and CAT values in rats given aloe vera. Singh et al. (30) in a study investigating the carcinogen-metabolizing phase I and phase II enzymes, antioxidant enzymes, glutathione content, lactate dehydrogenase, and lipid peroxidation in livers of mice fed two different doses of aloe vera showed that different doses of aloe vera had a protective effect against membrane and cell damage triggered by pro-oxidants. Similarly, in an experimental study conducted by Guven et al. (31) on the protection of aloe vera from sciatic nerve I/R injury, MDA in the aloe vera therapy group was statistically significantly lower than that of the SIR group. Additionally, SOD decreased more in the aloe vera therapy group than in the SIR group. This shows that aloe vera displays a protective effect in resolving oxidative damage caused by free radicals occurring due to different reasons in tissues. Nitric oxide synthase (NOS) is a unique enzyme in enzyme cell types and catalyzes NO synthesis. Three different isoforms are described for NOS: neural NOS (nNOS, type 1) (32), inducible NOS (iNOS, type 2) (33) and endothelial NOS (eNOS, type 3) (34, 35). This study used anti-iNOS staining to show the protective effect of aloe vera in renal and lung tissue.

A limiting factor in this study is that tissue injury in organs distant to I/R was not investigated using inflammatory cytokines. In fact, the anti-inflammatory properties of aloe vera are well-known. (21). In this study, a reduction in tissue damage was observed histopathologically and in tissue MDA.

Conclusion

This study indicated that aloe vera is protective against oxidative damage formed by I/R in distant organs like lungs and kidneys.

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Conflicts of interest

No potential conflicts of interest were disclosed.

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