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Research Article

An Investigation on Protective Effect of *Viburnum opulus* L. Fruit Extract Against Ischemia/Reperfusion-Induced Oxidative Stress After Lung Transplantation in Rats [1] [2]

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Abstract

The aim of this study was to investigate the protective effect of *Viburnum opulus* L. fruit extract against ischemia/reperfusion (I/R)-induced oxidative stress during the lung transplantation. For this purpose, 30 female rats were firstly randomized to form of donor and recipients. After then, the rats were divided into three groups named as control, I/R, and *V. opulus* + I/R. Experimental rats were subjected to lung transplantation with ischemia followed by 2 h of reperfusion. Transplantation-related lung injury was evaluated by multiple parameters. A significant decrease was observed in the enzyme activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), and the levels of glutathione and total antioxidant status (TAS), whereas the levels of malondialdehyde (MDA), total oxidant status (TOS), and protein carbonyl were significantly increased in lung tissue samples of I/R group in comparison to the control group. However, treatment with *V. opulus* fruit extract resulted in significant reduction of MDA and protein carbonyl levels and increment of the antioxidant system. In conclusion, *V. opulus* fruit extract showed protective effects against I/R-induced oxidative stress during lung transplantation probably by the radical scavenging and antioxidant activity. Therefore, this fruit extract can be efficient in the prevention of I/R-related lung toxicity.

Keywords: *Oxidative Stress, Transplantation, Ischemia/Reperfusion, Antioxidant*

Ratlarda Akciğer Transplantasyonda Iskemi/Reperfüzyonun İndüklediği Oksidatif Strese Karşı *Viburnum opulus* L. Meyve Ekstresinin Koruyucu Etkisinin Araştırılması

Özet

Bu çalışma, akciğer transplantasyonu sırasında iskemi/reperfüzyonun indüklediği oksidatif hasara karşı *Viburnum opulus* L. meyve ekstresinin koruyucu etkisini araştırmak amacıyla yapılmıştır. Bu amaçla, ilk olarak donör ve alıcı oluşturmak için 30 dişi rat rastgele seçildi. Daha sonra, ratlar kontrol, I/R ve *V. opulus* + I/R olarak adlandırılan üç gruba ayrıldı. Deneysel ratlara iskeminin ardından 2 saat reperfüzyon ile akciğer transplantasyonu yapıldı. Transplantasyon ile ilişkili akciğer hasarı, farklı parametrelerle incelendi. Kontrol grubu ile karşılaştırıldığında I/R grubunun akciğer doku örneklerinde süperoksit dismutaz (SOD), glutatyon peroksidaz (GPx) ve katalaz (CAT) enzim aktiviteleri, glutatyon ve total antioksidan durum (TAS) düzeylerinde önemli bir azalış, malondialdehit (MDA), total oksidan durum (TOS) ve protein karbonil düzeylerinde anlamlı artış belirlendi. Bununla birlikte, *V. opulus* meyve ekstresi ile uygulama sonrası MDA ve protein karbonil düzeylerinde önemli azalma ve antioksidan sistemde artma saptanmıştır. Sonuç olarak, akciğer transplantasyonu sırasında I/R'nin indüklediği oksidatif strese karşı *V. opulus* meyve ekstresinin muhtemel radikal temizleme ve antioksidan aktivitesi ile koruyucu etkilerinin olduğunu göstermiştir. Bu nedenle, bu meyve ekstresi I/R ilişkili akciğer hasarının önlenmesinde etkili olabilir.

Anahtar sözcükler: *Oksidatif Stres, Transplantasyon, İskemi/Reperfüzyon, Antioksidan*

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INTRODUCTION

Lung transplantation has become an effective therapeutic option in the treatment of patients with various end-stage pulmonary diseases [1] such as chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, cystic fibrosis, bronchiectasis, and primary pulmonary hypertension [2]. Lung injury induced by ischemia-reperfusion (I/R) can lead to severe primary graft failure with about 60% mortality after lung transplantation [3-5]. Although, there are significant improvements in surgical techniques, lung preservation, donor management and immunosuppressive strategies [6,7], I/R injury occurs in up to 22% of patients and is still the main cause of death within the first month of surgery [8]. I/R injury causes oxidative stress characterized by production of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, hydroxyl radicals, and hypochlorous acid [9,10], which potentially can be more detrimental than its local effects [11].

In recent times, there is an attention for the significance of antioxidant substances such as phenolic compounds present in the plants [12], which provide protection against toxic free radicals due to their radical scavenger activities and have therapeutically beneficial effects on human health [13].

Viburnum opulus L. is known as "Gilaburu" in Turkey and "Guilder rose" in Europe [14]. *V. opulus* growing in Turkey have been so far reported to possess several biological activities, including antioxidant, hepatoprotective, hypoglycemic, antinociceptive, and antiinflammatory effects [15]. It has been traditionally used to prevent the formation of kidney stone [16]. Phenolic acids such as hydroxybenzoic acids, tannins, coumarins, cathechols, iridoid glycosides, antocyanins, ascorbic acid, chlorogenic acid, salicin, (+)-catechin, (-)-epicatechin, cyanidin-3 glucoside, cyanidin-3-rutinoside, and quercetin [15-17] were determined in the composition of *V. opulus* fruit extract.

V. opulus fruit is known to have an important antioxidant effect, including strong radical scavenging activity [12,18]. Therefore, the objective of this study was to investigate the possible protective effects of *V. opulus* fruit extract against I/R-induced oxidative stress during the lung transplantation. Lung injuries were assessed by multiple parameters, including measurements of oxidative stress markers [superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), total glutathione, malondialdehyde (MDA), protein carbonyl, total antioxidant status (TAS), total oxidant status (TOS)], oxygenation index (arterial oxygen tension/inspired oxygen concentration ratio-PaO_2/FiO_2), wet/dry weight ratio of transplanted lungs, and histologic examination.

MATERIAL and METHODS

Chemicals

All reagents used in this study were analytical grade and obtained from Sigma Chemical Co. (St. Louis, MO, USA) and Merck (Darmstadt, Germany). TOS and TAS kits were purchased from Rel Assay Diagnostic (Turkey). Total glutathione and protein carbonyl kits were purchased from Cayman (Ann Arbor, MI, USA).

Plant Material and Preparation of Extract

V. opulus fruits were collected after full maturation in September 2014 from city of Kayseri in Turkey. Air-dried and powdered fruits (100 g) were macerated in methanol for 3 days. Sample was then filtered through Whatman No 1 papers in a Buchner funnel. The macerates were evaporated in-vacuo until dryness and lyophilized.

Estimation of Total Phenolic Content and Identification with LC-MS/MS

Total phenolic content was estimated quantitatively using the method described by Jindal and Singh [19]. A standard curve was prepared by using different concentrations (0.1-100 mg/mL) of gallic acid and used for the determination of total phenolic compounds content (mg gallic acid equivalent-GAE/g extract).

In order to identify the active phenolic compounds present in *V. opulus* aqueous methanol (70%) extract, UPLC-MS/MS with an electrospray ionisation (ESI) was utilized. UPLC-ESI-MS/MS analyses were performed on an UPLC instrument with Q-trap mass spectrometer (Shimadzu, Japan). The system was operated using LabSolutions software. The flow rate was 0.2 mL/min and mobile phase was mixture of A (1% acetic acid in water) and B (1% acetic acid in methanol). Ultrahigh pure helium (He) and high purity nitrogen (N_2) were used as collision and nebulising gases, respectively. The mass spectrometer was operated in negative ionisation mode, and mass spectra were recorded between 100 and 1.000 amu.

Animals and Experimental Groups

All procedures performed on animals were in accordance with the European Union Directive 2010/63/EU for care and use of laboratory animals. The experiment protocol was approved by the Ethical Committee for Animal Research at Erciyes University (Approval date: 13.03.2013; no: 13/52). Thirty female Wistar albino rats (weighing 250-300 g) were used in our study. They were housed in individual cages under standard laboratory conditions (12 h/12 h light/dark cycle, 22-24°C temperature and 55-60% relative humidity). A commercial pellet diet (2.600 kcal/kg metabolic energy, 7% crude fiber and 23% crude protein) and fresh drinking water were given *ad libitum*.

At the beginning, 30 female Wistar albino rats were randomized to form of donor (n=12) and recipients (n=18). After then, the rats were divided into groups as follows; control group (n=6), I/R (n=6), and *V. opulus*-treated I/R (VO+I/R) (n=6). In the control group, we obtained samples from the left lung lower lobe without any additional procedure for biochemical and histopathological evaluation.

Surgical Procedures

Lung transplantation was performed as reported previously by Yucel et al.[20]. In the donor group, left upper lobectomy was performed and the left lower lobe was removed as an allograft with the following procedures: The rats were anesthetized with an intraperitoneal injection of 75 mg/kg ketamine and 10 mg/kg xylazin combination. The rats were shaved after induction and fixed in a supine position. Each rat was heparinized intravenously (15 U). Following midline incision, tracheostomy was performed. Ventilation was maintained with tidal volume of 1.5 mL room air at 60 breaths/min and the positive end expiratory pressure (PEEP) of 2 cm-H_2O. Median sternotomy incision was used for opening the thoracic cavity. Left pulmonary artery, vein and bronchus were exposed by dissecting left hilar region. The upper lobe branches of pulmonary artery and vein were tied with 5/0 free silk suture and cut. The left upper lobe bronchus was tied with 4/0 free silk suture and cut. So, left upper lobectomy was completed. Following left upper lobectomy, pulmonary artery and vein of left lower lobe were catheterized in the peripheral direction. Placed catheters were fixed with 4/0 free silk suture and then left lower lobe artery, vein, and bronchus were tied and cut. After completion of the removal of allograft lung tissue, a catheter was placed in the left lower lobe bronchus to provide a three-way flow. The first line was fixed in the left lower lobe bronchus. The second line is attached to the intrabronchial pressure gauge. The last line was fixed to ventilation balloon. The washing process of the left lower lobe was performed by giving Euro-Collins solution at pressure of 1.5 cm H_2O to artery catheter of left lung lower lobe. During the washing process, it was manually reventilated with not exceeding 0.2 mL of room air, 60 breathes/min, and PEEP of 1.5 cm H_2O from the left lower lobe bronchus. The allograft lung tissue was immersed in the +4°C Euro-Collins solution for 24 h in a half-inflated manner. Donor group were sacrificed by infusing a lethal dose of anesthetics afterwards. Six of the donor lungs were reperfused and reventilated in I/R group and the other six were reperfused and reventilated in VO + I/R group.

I/R Group: Left lower lobectomy was performed in I/R group, after left upper lobectomy. Thus, left pneumonectomy was completed. Unlike the subject of the donor group, pulmonary artery and vein were catheterized in the proximal direction. Placed catheters were fixed with 4/0 free silk suture and then left lower lobe of artery, vein, and bronchus were tied and cut. Thus, the catheterization was completed. After catheterization, the allograft left lower lobe was reventilated and reperfused for two hours. Catheter in the pulmonary artery of lung obtained from donor was combined with the placed one in the pulmonary artery of left lung lower lobe. Again, catheter in the pulmonary vein of left lower lobe obtained from donor was combined with the placed one in the pulmonary vein of left lung lower lobe. After the anastomosis process, reperfusion was allowed. Simultaneously, the animals were manually reventilated not to exceed 1.2 mL of room air, 60 breaths/min, and PEEP pressure of 1.5 cm H_2O with the help of ventilation device. Also, allograft lung tissue was manually reventilated not to exceed 0.2 mL of room air, 60 breathes/min, and PEEP pressure of 1.5 cm H_2O with the help of ventilation device. The reperfusion and ventilation was continued for 2 h. After 2 h, the allograft lungs were sampled for histopathological and biochemical tests.

VO+I/R Group: After the left upper lobectomy as in I/R group, the subjects' left lower lobe pulmonary artery and vein were catheterized. Unlike subjects in I/R group, *V. opulus* fruit extract (200 mg/kg) was administered intraperitoneally to both this group (the recipient) and six subjects of the donors group 3 h before anesthesia [3]. Immediately after the lung transplantation, recipients was given again the same dose of the fruit extract intraperitoneally. The rats were reperfused and reventilated as in the I/R group and tissue sampling was performed for pathologic and biochemical examinations.

Tissue and Blood Sample Collection and Preparation

After 2 h reperfusion, blood was sampled from the right carotid artery after 10 min of ventilation, followed by immediate blood gas analysis. Blood samples were centrifuged at 1.000 g for 10 min to separate plasma. The plasma samples were stored at -80°C until analysis of TAS, TOS, and protein carbonyl using ELISA reader (Biotek Synergy HT, Vermont, USA).

Lung samples were homogenized in ten volumes of ice-cold 140 mM KCl and Tris-HCl buffer (50 mM, pH 7.6) using a homogenizer (IKA Ultra-Turrax T10 basic model, Germany) for 2 min at 13.000 rpm. The total glutathione and MDA levels were determined in the tissue homogenates by using spectrophotometer (UV-1800, Shimadzu Co., Kyoto, Japan). The homogenates were then centrifuged at 5.000 g for 60 min to remove debris. Clear supernatant was used for SOD, GPx, and CAT enzyme activity assays. Protein content in tissue homogenate and supernatant was measured according to the method of Lowry et al.[21].

Analyses of Oxidative Stress Parameters

SOD activity was measured as described by Fitzgerald et al.[22]. Briefly, the tissue samples were diluted with 10 mM phosphate buffer, pH 7.0. 25 µL aliquots were mixed with 850 µL of substrate solution containing 0.05 mmol/L xanthine sodium and 0.025 mmol/L

2-(4-iodophenyl)-3-(4-nitrophenol) 5-phenyltetrazolium chloride (INT) in a buffer solution containing 50 mmol/L CAPS (3-(cyclohexylaminol)-1-propanesulfonic acid) and 0.094 mmol/L EDTA (pH 10.2). To the mixture, 125 µL xanthine oxidase (80 U/L) was added and then the increase of absorbance was recorded by spectrophotometer at 505 nm for 3 min. SOD activity was expressed in U/mg protein.

GPx activity was measured as previously described by Pleban et al.[23]. Briefly, a reaction mixture containing 1 mmol/L Na_2EDTA, 2 mmol/L reduced glutathione, 0.2 mmol/L NADPH, 4 mmol/L sodium azide and 1.000 U glutathione reductase in 50 mmol/L Tris buffer (pH 7.6) was prepared. 20 µL of tissue samples and 980 µL of the reaction mixture were mixed and incubated for 5 min at 37°C. The reaction was initiated by adding 8.8 mmol/L hydrogen peroxide (H_2O_2) and the decrease of absorbance recorded at 340 nm was monitored by spectrophotometer for 3 min. GPx activity was expressed in U/mg protein.

CAT activity was measured in tissue samples at 25°C by the method of Aebi [24]. The decomposition rate of the substrate H_2O_2 was monitored spectrophotometrically at 240 nm for 30 sec. The activity was expressed as U/mg protein.

Lipid peroxidation was estimated by measurement of MDA in tissue samples by the method described by Ohkawa et al.[25]. After reaction of MDA with thiobarbituric acid reactive substances (TBARS), the reaction product was followed spectrophotometrically at 532 nm, using tetramethoxypropane as a standard. The results were expressed as nmol/mg protein.

For the estimation of glutathione (GSH) levels in tissue samples, a commercial kit based on the enzymatic recycling method was utilized. The assay was based on the reaction of sulfhydryl group of glutathione with 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB, Ellman's reagent) resulting in the production of 5-thio-2-nitrobenzoic acid (TNB). The disulfide formed between GSH and TNB was then reduced by glutathione reductase to recycle the GSH and produce TNB. Since the rate of TNB production is directly proportional to concentration of GSH in the sample, the absorbance of TNB was measured at 412 nm to evaluate total glutathione. Tissue homogenates were deproteinated using 10% metaphosphoric acid and pH was adjusted with 4M triethanolamine before assay. The results were expressed as µmol/g tissue.

Protein carbonyl levels were analyzed in the plasma samples based on the reaction between 2,4-dinitrophenylhydrazine and protein carbonyls using a commercially available kit. The absorbance of resulting protein-hydrazone was measured at 370 nm as described by the manufacturer. Following standardization to the related protein concentration for each sample, the carbonyl content was expressed as nmol/mg protein.

TAS and TOS assays developed by Erel [26] were carried out by commercially available kits in plasma samples. Principle of the assay for TAS levels is as follows; anti-oxidants in the samples reduce dark blue-green colored 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical to colorless reduced ABTS form. The change of absorbance at 660 nm is related with total antioxidant level of the sample. The results were expressed as µmol Trolox equivalent (eq)/mg protein. TOS measurement is based on the oxidation of the ferrous ion-o-dianisidine complex to ferric ion by the oxidants present in the sample. The ferric ion forms a colored complex with xylenol orange in an acidic medium. The color intensity, measured spectro-photometrically, is related to the total amount of oxidant molecules present in the sample. The results were expressed µmol H_2O_2 eq/mg protein. The percent ratio of TOS to TAS was used as OSI, an indicator of the degree of oxidative stress. The OSI value was calculated as follows: OSI = [(TOS, µmol H_2O_2 eq/mg protein)/(TAS, µmol Trolox eq/mg protein) x 100].

Oxygenation Index (PaO_2/FiO_2)

Oxygenation index was measured by blood gas analysis of blood samples from either the right carotid artery or the left pulmonary vein.

Wet/Dry Weight Ratio of Transplanted Lungs

Transplanted left lungs removed at 2 h after the reperfusion were sliced into 3 parts. After the upper one third was weighed immediately, it was dried at 80°C for 48 h to calculate the wet/dry weight ratio.

Histologic Examination

A tissue sample from the middle of the transplanted lung was fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin. The severity of lung injury was scored based on alveolar edema and congestion, interstitial edema and congestion, neutrophil infiltration. Histopathological changes were graded as follows: (0) absent, (1) mild, (2) moderate, (3) severe injury, respectively. For each lung tissue sample, the final value was the mean of the scores for the 3 separate slides [27].

Statistical Analysis

Analysis of the data was performed by using Statistical Package for the Social Sciences (SPSS version 18.0 for Windows, Chicago, IL, USA). The comparison of the results among different groups was carried out by one-way ANOVA and followed by Tukey multiple comparisons test. The results were expressed as aritmetic mean±standard deviation (SD). The values were considered statistically significant if the P value was less than 0.05.

RESULTS

Total Phenolic Content and Identified Compounds of V. opulus Fruit Extract

In fruit extract, total phenolic amount was found as 67.73 mg GAE/g extract.

Compound 1-8 were identified based on the MS fragmentation data in negative ionisation mode compared with literature [28,29] as shown in *Table 1*.

Oxidative Stress Parameters

As presented in *Fig. 1A*, the SOD activity in group of I/R (1.65±0.33 U/mg protein) was significantly lower than that in groups of control (3.6±0.37 U/mg protein) and VO+I/R (3.31±0.43 U/mg protein) (P<0.05). However, treatment with *V. opulus* markedly improved this effect as compared with I/R group (P<0.05). The GPx activity in I/R group (1.42±0.29 U/mg protein) was markedly lower than that of the control (3.18±0.25 U/mg protein) group and VO+I/R (2.84±0.28 U/mg protein) group (P<0.05). However, this effect was significantly ameliorated by *V. opulus*-treatment as compared to I/R group (P<0.05) as presented in *Fig. 1B*. There was a significant depletion in the activity of CAT in group of I/R (17.97±1.62 U/mg protein) compared to control (27.02±1.41 U/mg protein) and VO+I/R (24.5±1.72 U/mg protein) groups (P<0.05) as demonstrated in *Fig. 1C*. I/R resulted in a significant decrease of glutathione levels (1.32±0.43 µmol/g tissue) compared with control group

Table 1. *Identified compounds of V. opulus fruit extract by LC-MS/MS*

No	Molecular Mass	[M-H]⁻ m/z	Compound	Amount (%)*
1	133	115, 71	Malic acid	24.18
2	179	89, 68	Caffeic acid	3.31
3	191	111, 85	Quinic acid	5.31
4	295	179, 133, 89	Caffeic acid derivative	27.09
5	337	277, 174	Coumaroyl-quinic acid	1.64
6	353	191	Chlorogenic acid	5.36
7	451	341, 133	Not identified	1.91
8	613	295, 133, 89	Not identified	2.01

** Relative amounts from total ion chromatogram*

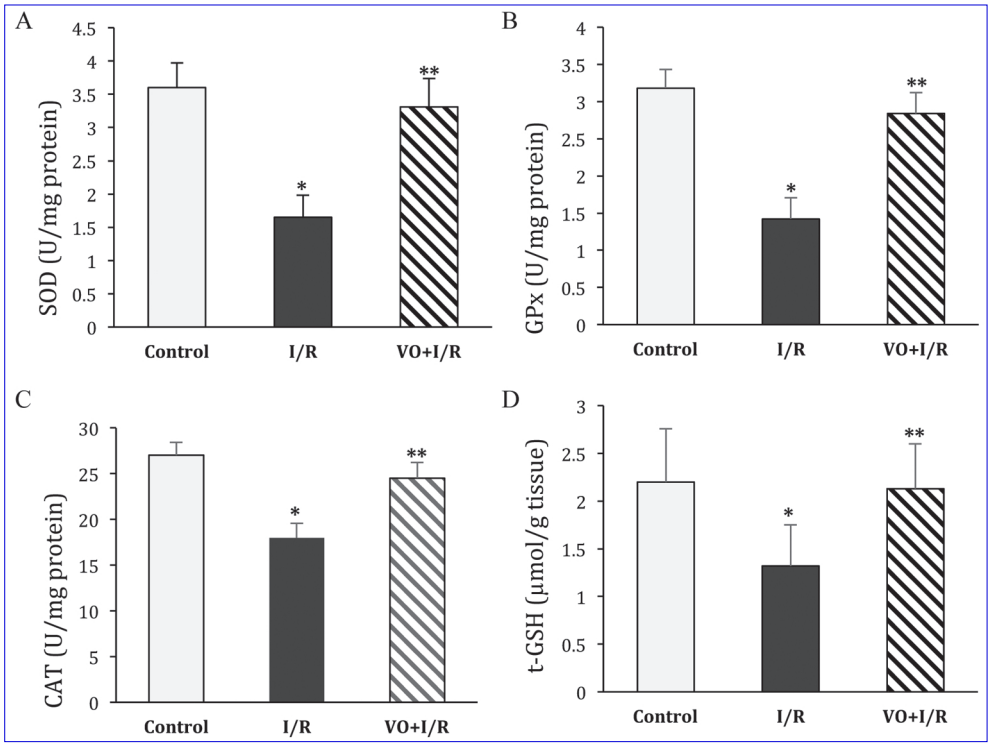

Fig 1. Effects of *V. opulus* fruit extract treatment on (A) SOD, (B) GPx, (C) CAT enzyme activities, and (D) t-GSH. Values are indicated by mean±SD. * P<0.05 vs. control group; ** P<0.05 vs. I/R group

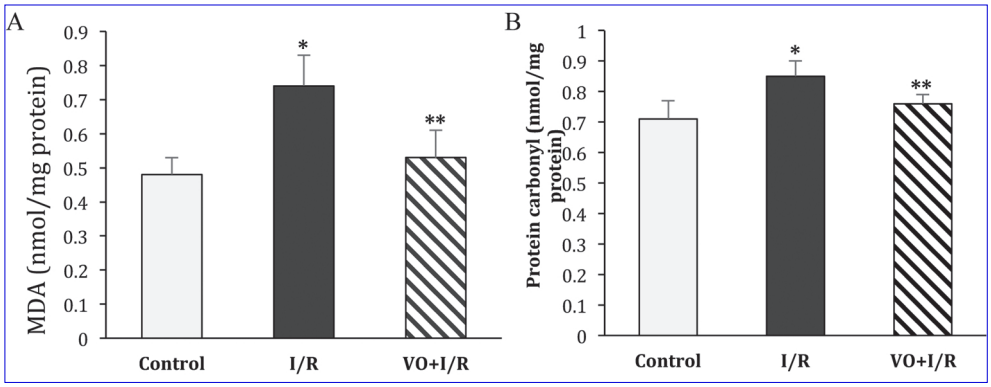

Fig 2. Effects of *V. opulus* fruit extract treatment on (A) MDA and (B) protein carbonyl levels. Values are indicated by mean±SD. * P<0.05 vs. control group; ** P<0.05 vs. I/R group

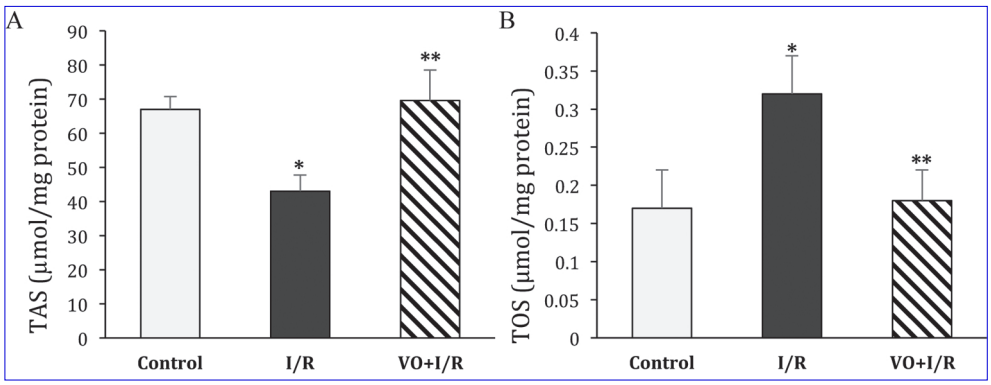

Fig 3. Effects of *V. opulus* fruit extract treatment on (A) TAS and (B) TOS levels. Values are indicated by mean±SD. * P<0.05 vs. control group; ** P<0.05 vs. I/R group

Table 2. *The comparison of the parameters among the groups*

Parameters	Control (mean±SD)	I/R (mean±SD)	VO+I/R (mean±SD)
PaO_2/FiO_2	454±18.64	354±32.54*	386±12.53*
Wet/dry weight ratio	4.98±0.23	5.66±0.91	5.39±0.69
Histological score	0.83±0.98	2.16±0.40**	0.16±0.40

** P<0.05 vs. control group; ** P<0.05 vs. VO+I/R*

(2.20±0.56 µmol/g tissue), while this effect was efficiently compensated by *V. opulus* treatment (2.13±0.47 µmol/g tissue) (P<0.05) as shown in *Fig. 1D*.

Fig. 2A shows that the lung tissue levels of MDA in I/R group (0.74±0.09 nmol/mg protein) were significantly higher than that in the control group (0.48±0.05 nmol/mg protein) and VO+I/R group (0.53±0.08 nmol/mg protein). However, *V. opulus* fruit extract treatment resulted in a significant reduction in MDA levels as compared to I/R group (P<0.05). As expected, the plasma protein carbonyl level was significantly elevated in I/R group (0.85±0.05 nmol/mg protein) as compared with control (0.71±0.06 nmol/mg protein), and we observed that *V. opulus* fruit extract-treatment counteracted this effect (0.76±0.03 nmol/mg protein) *(Fig. 2B*, P<0.05).

Our results indicated that plasma TAS levels were significantly lower (P<0.05) in the group of I/R (43.02±4.75 µmol Trolox eq/mg protein) as compared to groups of control (66.98±3.8 µmol Trolox eq/mg protein) and VO+I/R (69.59±8.9 µmol Trolox eq/mg protein) as presented in *Fig. 3A*. We observed that plasma TOS levels were significantly higher (P<0.05) in I/R group (0.32±0.05 µmol H_2O_2 eq/mg protein) as compared with groups of control (0.17±0.05 µmol H_2O_2 eq/mg protein) and VO+I/R (0.18±0.04 µmol H_2O_2 eq/mg protein), while administration of *V. opulus* fruit extract significantly decreased the TOS levels in VO+I/R group as shown in *Fig. 3B*. The comparison of control and I/R groups in terms of OSI% did reveal a statistically significant difference between these groups (P<0.05). The similar results were also observed between I/R and VO+I/R groups.

Oxygenation Index (PaO_2/FiO_2)

As shown in *Table 2*, compared with the control group, the PaO_2/FiO_2 level was significantly decreased by 22% at 2 h after reperfusion in I/R group (P<0.05). However, when compared with I/R group, administration of *V. opulus* fruit extract was able to elevate the PaO_2/FiO_2 level by 9% (P<0.05).

Wet/Dry Weight Ratio of Transplanted Lungs

As an indicator of pulmonary edema, wet/dry weight ratios were calculated. As demonstrated in *Table 2*, it was seen a slight increase by 14% in wet/dry weight ratio in group of I/R compared with the control group, but a statistical association was not detected. On the other hand, there was a slight decrease level by 5% in wet/dry weight ratio in group of VO+I/R compared with I/R group.

Histopathological Findings

Histological examination of the lung tissue subjected to I/R process showed the distinctive pattern of ischemic injury, which included interstitial distinct congestion and edema, widespread edema and congestion in the alveoli. In the control group, lung tissue sections had a normal morphology. As indicated in *Table 2*, treatment with *V. opulus* fruit extract significantly decreased the histological injury score in VO+I/R group compared with I/R group ($P<0.05$).

DISCUSSION

In our study, I/R injury resulted in decrease of SOD, GPx and CAT enzyme activities as well as glutathione levels as compared with control group, while this effect was compensated by *V. opulus* treatment. In line with our findings, a report of an experimental model has shown the beneficial effect of *V. opulus* treatment on the levels of total thiols and glutathione [30]. MDA elevation shows increased lipid peroxidation due to the interaction between lipid components of cellular membranes and ROS [31]. In the present study, we indicated that MDA level was increased in I/R group. Administration of *V. opulus* fruit extract resulted in significant reduction of tissue MDA levels in VO+I/R group compared with I/R group. In this study, we carried out total oxidant and antioxidant status at the same time to more accurately evaluate oxidative stress. We observed that plasma TOS levels were significantly higher and TAS levels were significantly lower in I/R group as compared to groups of control and VO+I/R. The comparison of control and I/R groups in terms of OSI did reveal a statistically significant difference between these groups. The similar results were also observed between I/R and VO+I/R groups.

Similar to other biomolecules, proteins are prone to attacks during oxidative stress conditions. In this context, protein carbonyls represent an irreversible form of protein modification and are relatively stable; therefore determination of protein carbonyl levels serves as an overall marker of protein oxidation [32]. Our study has revealed the beneficial effect of *V. opulus* fruit extract-treatment in I/R-induced oxidative stress as evidenced by the compensation of the increase in plasma protein carbonyl level.

Transplantation-related lung injury was showed by decreased levels of PaO_2/FiO_2. The level of PaO_2/FiO_2 was significantly decreased at 2 h after reperfusion in I/R group compared with the control group. However, administration of *V. opulus* extract prevented the decrease in PaO_2/FiO_2 level. Sun et al.[3] found similar result with curcumin at the early stage of post-transplantation.

In our study results were in agreement with the previous ones. Sun et al.[3] suggested that curcumin, which is an active component of *Curcuma longa*, can be an alternative therapy for protecting lung transplantation-related I/R injury. They found that there was a significant increase in MDA level and a significant decrease in TAS levels in I/R group compared with the control group. Pretreatment with curcumin significantly prevented the increase in MDA level and the decreased TAS level. In our previous study [1], we demonstrated that donor treatment with taurine protected the lungs of rats against post-transplantation I/R injury in respect to histopathological and biochemical findings. Following treatment with taurine, we observed increased activities of SOD and CAT, and decreased MDA levels in I/R group compared with taurine-treatment group.

It was found that *V. opulus* fruit extract has strong radical scavenging activity and antioxidant effects due to its phenolic substance contents [12,16,18]. We also determined the active phenolic compounds such as caffeic acid derivative and malic acid in our *V. opulus* fruit sample. Our results indicated that *V. opulus* fruit extract administration to animals before/during lung transplantation significantly reduced I/R-related lung injury by preventing oxidative damage. These results may explain that the active compounds determined with LC-MS/MS in *V. opulus* fruit extract provided the preventive effect on oxidative stress. Some herbal drugs have been used to prevent certain diseases due to their capacity of radical scavenging activity [12]. Liu et al.[33] showed that *Ginkgo biloba* extract had a protective effect on lung injury induced by I/R, which may be related to its antioxidant property. *Ginkgo biloba* extract markedly increased SOD activity, reduced MDA levels. He et al.[34] suggested the protective effects of triptolide (Tripterygium extracts of the Chinese herb) on I/R-induced injury of transplanted rabbit lungs. Zayachkivska et al.[35] also identified that pretreatment with *V. opulus* proanthocyanidins exhibited a potent gastroduodenoprotective effect by the suppression of lipid peroxidation and an enhancement of SOD, CAT activities in rats.

In conclusion, our findings showed that *V. opulus* xtract indicated protective effects against I/R-induced oxidative stress during lung transplantation probably by the radical scavenging and antioxidant activities. Therefore, *V. opulus* fruit extract can be alternative therapeutic strategy for the prevention of I/R-related lung toxicity.

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