

Possible protective effect of pomegranate peel powder on lung injury induced by skeletal muscle ischemia-reperfusion

Original
Article

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ABSTRACT

Introduction: Multi-organ failure with life-threatening sequel occurs in a variety of surgical practices as a result of ischemia-reperfusion (I/R) injury.

Aim of work: In this study, more light is thrown on the histological lung changes induced by skeletal muscle I/R with reference to their underlying mechanisms and to determine, for the first time, the protective role of pomegranate peel powder on the lung injury induced by skeletal muscle I/R.

Materials and Methods: Thirty adult male albino rats were divided equally into three groups: sham control group, I/R group and I/R group pretreated with pomegranate peel powder (500 mg/kg dissolved in distilled water twice daily orally for 10 days). At the end of the experiment, blood samples were obtained to assess serum malondialdehyde (MDA) then lung specimens were processed for routine histological examination and detection of collagen deposition. Myeloperoxidase (MPO), a marker of neutrophils infiltration, transforming growth factor- β (TGF- β), an inflammatory and pro-fibrotic mediator and Bcl2, an anti-apoptotic marker, were detected immunohistochemically.

Results: Loss of general architecture of the lung tissues, massive inflammatory infiltrate, emphysema, edema, congested blood vessels, thickened inter-alveolar septa, destruction of some alveoli, disorganization and focal sloughing of the bronchial epithelium in addition to increased collagen deposition were detected in I/R group. Oxidative stress in the form of significant increase of MDA level in addition to up-regulation of both MPO and TGF- β and down-regulation of Bcl2 were implicated in the pathogenesis of acute lung injury induced by skeletal muscle I/R. Pomegranate peel powder significantly ameliorated these damaging effects through its antioxidant, anti-inflammatory, anti-fibrotic and anti-apoptotic effects.

Conclusion: Pretreatment of rats with pomegranate peel protected the lungs against skeletal muscle I/R injury.

Received: 01 May 2018, **Accepted:** 11 September 2018

Key Words: Ischemia-Reperfusion, lung, pomegranate peel.

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ISSN: 1110-0559, Vol. 41, No. 2

INTRODUCTION

With increasing incidence of either musculoskeletal trauma or malignant tumor, the need to reconstructive surgery techniques that preserve these affected regions is also in increase. Unfortunately, ischemia reperfusion (I/R) injury remains one of the major causes of multi-organ failure that may lead to fatal complications^[1].

Ischemia/reperfusion (I/R) injury occurs as a result of interruption of the blood flow with subsequent reperfusion leading to significant tissue damage^[2]. Release of toxic metabolites and several inflammatory mediators into the systemic circulation occurs as a result of reperfusion of ischemic tissues than ischemia itself^[3]. In addition, apoptotic changes, dysfunction of anti-oxidant mechanisms, and injury of the protein, lipids, deoxyribonucleic acid (DNA) and mitochondria are incorporated in multi-organ damage and even failure^[4]. One of the most important target organs in multiple organ failure caused by severe injury is the lung^[5].

Anti-oxidants may limit I/R induced injury in humans^[6]. One of the important dietary sources of antioxidants is the pomegranate (*Punicagranatum* L.), belonging to the family Punicaceae^[7]. Pomegranate has high antioxidant capacity and total polyphenol contents including ellagic acid, gallagic acid, punicalin, and punicalagin^[8]. The peel is an important source of bioactive compounds such as phenolics, flavonoids, ellagitannins, and proanthocyanidin compounds, complex polysaccharides and minerals mainly potassium, nitrogen, calcium, phosphorus, magnesium and sodium^[9]. Punicalagin is the most abundant polyphenol and is mainly found in pomegranate peel^[10].

This study aims to determine, for the first time, the possible protective role of pomegranate peel powder on the lung injury induced by skeletal muscle I/R.

MATERIALS AND METHODS

Fresh pomegranate fruits were collected from the market and cleaned. The pomegranate peels were isolated, divided into small pieces and air dried. The dried peels

were then grinded into fine powder that was stored in a clean airtight container^[11].

Thirty adult male albino rats, weighing 150-200 gms each, were used in this study. They were purchased from and the experiment was done in the animal house of Medical Research Institute, Alexandria University, Egypt. This study was conducted according to the guidelines of the animal care review board of the Faculty of Medicine, Menoufia University, Egypt and adhering to the guide for care and use of laboratory animals and the study was approved by the ethics committee.

Experimental groups:-

The rats were maintained under constant room temperature and standard conditions with ad libitum access to water and commercial food and were placed in individual plastic cages with soft bedding. Rats were divided equally into three groups of ten rats each:

Group I (sham control group):- The rats were subjected to all operative procedures, except arterial occlusion and reperfusion.

Group II (ischemia-reperfusion group):- The rats were subjected to 2 hours of ischemia followed by 24 hours of reperfusion.

Group III (ischemia-reperfusion + pomegranate peel group):- The rats in this group were pretreated with pomegranate peel powder (500 mg/kg body weight) that was dissolved in distilled water and given by oral intubation twice a day for 10 days before ischemia. On post-treatment day 11, rats underwent 2 hours hind limb ischemia by clamping the femoral artery, then the clamp was removed and the rat underwent 24 hours reperfusion.

Surgery:-

Anesthesia was induced using ketamine plus xylazine (10 mg/kg and 50 mg/kg i.m., respectively). After induction of anesthesia, the left hind limb was completely clipped, disinfected and a skin incision was made on the medial surface of the left hind limb. The femoral artery was isolated and clamped with a vascular clamp for 2 hours. The rats were kept anesthetized throughout the duration of the ischemic period and body temperature was maintained with a heating pad over the body. Following the ischemic period, the vascular clamp was removed and then the surgical site was routinely closed. After the surgery, fluid losses were replaced by intraperitoneal administration of 5 ml warm isotonic saline and the rats were returned to their cages with ad libitum access to water and commercial food during the reperfusion period.

After 24 hours of reperfusion, the rats were anesthetized with an overdose of intraperitoneal pentobarbital injection (300 mg/kg) and the intracardiac blood samples were collected for biochemical analysis then the lungs were removed en bloc and fixed in 10% neutral buffered formalin solution for histological and immunohistochemical examinations under light microscopy.

Biochemical analysis

Serum was obtained from the intracardiac blood samples by centrifugation. The serum concentration of malondialdehyde (MDA) is generally used as biomarker of lipid peroxidation that is used as an indicator of reactive oxygen species (ROS) mediated damage.

Histological examination

A midline thoraco-abdominal incision was performed to all rats and the lungs were removed and fixed in a 10% neutral buffered formalin solution then processed routinely by embedding in paraffin and sectioned into 5 µm thick sections to be stained with hematoxylin and eosin for routine histological assessment and masson trichrome stain for detection of collagen deposition.

The severity of the lung injury was graded into four grades as follows: grade 0, no diagnostic change; grade 1, mild neutrophil leukocyte infiltration and mild to moderate interstitial congestion; grade 2, moderate neutrophil leukocyte infiltration, perivascular edema formation and partial destruction of pulmonary architecture; and grade 3, dense neutrophil leukocyte infiltration and complete destruction of pulmonary structure^[12]. A total of five slides from each lung sample were randomly screened and the mean was accepted as the representative value of the sample.

Immunohistochemistry

Analysis of Myeloperoxidase (MPO), Bcl2 and transforming growth factor-β (TGF-β):-

The paraffin sections on poly-L-lysine coated slides were deparaffinized and rehydrated. Endogenous peroxidase was blocked by inserting the sections in 3% hydrogen peroxide (H₂O₂). The microwave antigen retrieval procedure was performed. The sections were incubated with primary anti MPO antibody (rabbit polyclonal, Lab Vision, USA), anti Bcl2 antibody (rabbit polyclonal, Abcam) and anti TGF-β antibody (mouse monoclonal, Abcam). After that, biotinylated goat-polyvalent secondary antibody was applied. The sections were then incubated in preformed streptavidin peroxidase and finally the prepared DAB substrate chromogen (3,3'-diaminobenzidine tetrahydrochloride) was applied and the slides were counterstained with hematoxylin to be examined under light microscope. This technique was performed in the Pathology Department, Faculty of Medicine, Menoufia University, Egypt.

Morphometric analysis

By using Image J program, version K 1.45, thickness of inter-alveolar septa, percentage of surface area of collagen deposition in addition to percentage of positive MPO, Bcl2 and TGF-β immunoreactive cells were measured.

For each parameter, ten non-overlapping fields ("x 20 for measuring the thickness and surface area" and "x 100

for counting the immunohistochemical positive cells”) for every specimen from five different rats/experimental group were examined. This was done in the Anatomy Department, Faculty of Medicine, Menoufia University, Egypt.

Statistical analysis

The data were collected, tabulated and analyzed by SPSS (statistical package for social science) version 17.0 on IBM compatible computer (SPSS Inc., Chicago, IL, USA). A *p* value of < 0.05 was considered statistically significant. A *p* value of < 0.001 was considered statistically highly significant and *P* value > 0.05 was considered non-significant. This was done in the Public Health Department, Faculty of Medicine, Menoufia University, Egypt.

RESULTS:

Biochemical results:

Serum level of MDA was significantly higher in I/R group than both control and protected groups. Moreover, the protected group showed significant increase in MDA level when compared to the control group (Table 1, Histogram 1).

Histological results:

Hematoxylin and Eosin stain (HxandE):-

Light microscopic study of HxandE stained sections from the sham control group showed normal lung tissue with clear alveoli, thin inter-alveolar septa, blood vessels and normal bronchial epithelial lining (Fig. 1).

Ischemia reperfusion group (I/R) revealed loss of general architecture of the lung tissues, distorted appearance of lung parenchyma, massive inflammatory infiltrate, emphysema, edema, destruction with narrowing of some alveoli and compensatory dilatation of others. Moreover, congested blood vessels, deposition of brown particles within the lung tissue most probably hemosiderin deposition as a result of breakdown of red blood cells, thickening of inter-alveolar septa and destruction of others in addition to disorganization and focal sloughing of the bronchial epithelial lining were observed (Fig 2).

Pretreatment by pomegranate peel powder decreased the lung changes to a great extent. Lung parenchyma was more or less with normal architecture except for mild inflammatory infiltrate. Sloughing of small areas of the bronchial epithelium was also noted (Fig 3).

Statistically, the thickness of the inter-alveolar septa was significantly higher among I/R group than control and protected groups. Furthermore, there was a non-significant difference between both the control and protected groups (Table 2 and Histogram 2).

Histopathology and lung injury score

Grades 2 and 3 of lesions were obtained in I/R group (as seen morphologically in Fig. 2), with an average of

2.6. These pathological changes in I/R group pretreated with pomegranate peel powder were much less than in I/R group (as seen morphologically in fig.3) with an average 0.8. There was a highly significant difference in the lung injury score between I/R group and both the control and protected groups with no statistical significance difference between the control and protected groups (Table 3).

Masson trichrome stain

Comparing to the control group, light microscopic study of masson trichrome stained sections showed moderate to massive increase in the collagen deposition within the lung tissue in I/R group that was decreased in the protected group (Fig. 4).

Statistically, there was a highly significant increase in the percentage of the surface area of collagen deposition in I/R group when compared to both control and protected groups. Moreover, there was a significant difference between both the control and protected groups (Fig 4, Table 4).

Immunohistochemical results:

The immunohistochemical results were detected by brown discoloration either cytoplasmic or nuclear.

Myeloperoxidase (MPO) immunoreaction:

The immunohistochemical results revealed an increase in the MPO immunoreaction, an indicator of neutrophilic infiltration, in I/R group compared with both control and I/R group pretreated with pomegranate peel powder. Statistically, the percentage of MPO positive cells was significantly higher in I/R group than both control and protected groups. Moreover, there was a non-significant difference between the control and protected groups (Fig 5, Table 5).

Bcl2 immunoreaction:

The results showed a decrease in the Bcl2 immunoreaction, an anti-apoptotic marker, in I/R group. This was partially relieved in I/R group pretreated with pomegranate peel powder. Statistically, the percentage of Bcl2 positive cells was significantly lower in I/R group than the other two groups. Furthermore, a non significant difference between both control and protected groups was observed (Fig 6, Table 5).

Transforming growth factor- beta (TGF-β) immunoreaction:

Regarding the TGF-β, there was an increase in the number of positive cells in I/R group in comparison to the other groups. This was partially improved in I/R group pretreated with pomegranate peel powder. Statistically, there was a significant increase in the percentage of TGF-β positive cells in I/R group when compared to the other two groups. Moreover, there was a significant difference between the control and protected groups (Fig 7, Table 5).

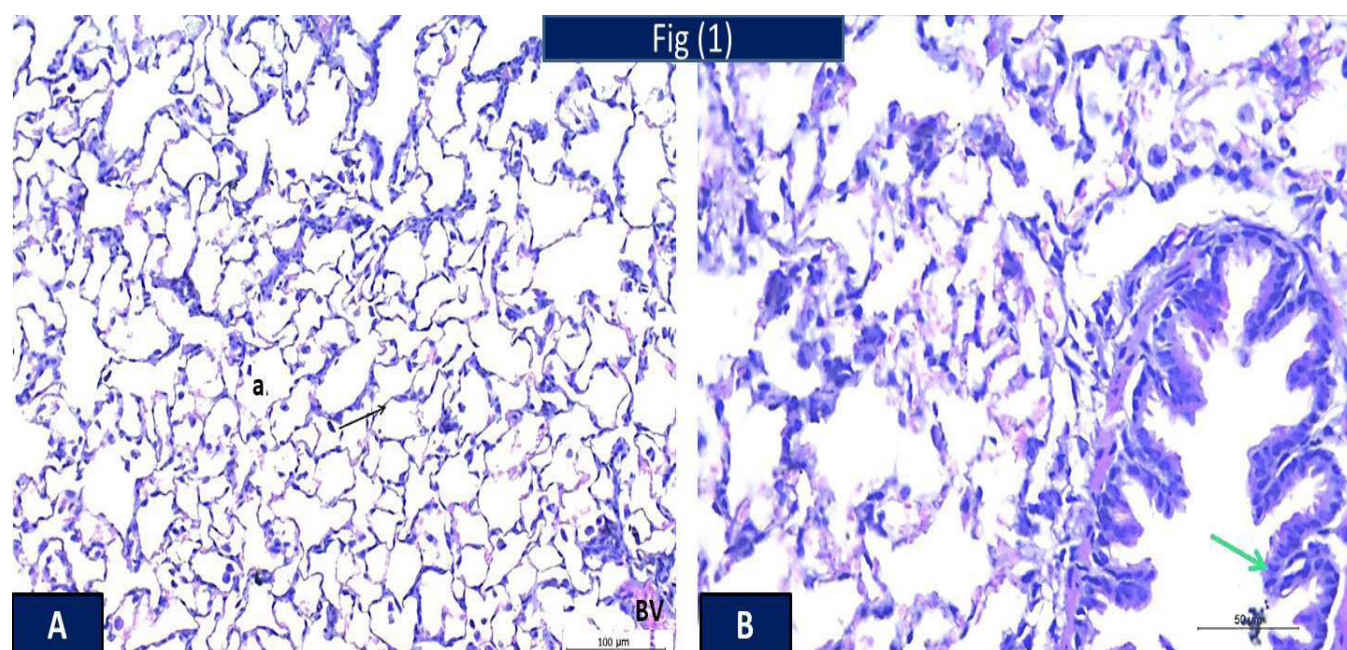


Fig 1: Photomicrographs of the control group:- (A): showing normal lung architecture with clear alveoli (a), blood vessels (BV) and thin inter-alveolar septa (black arrow) (HxandE X 20). (B): Normal bronchial epithelial lining (green arrow) is clearly seen (HxandE X 40).

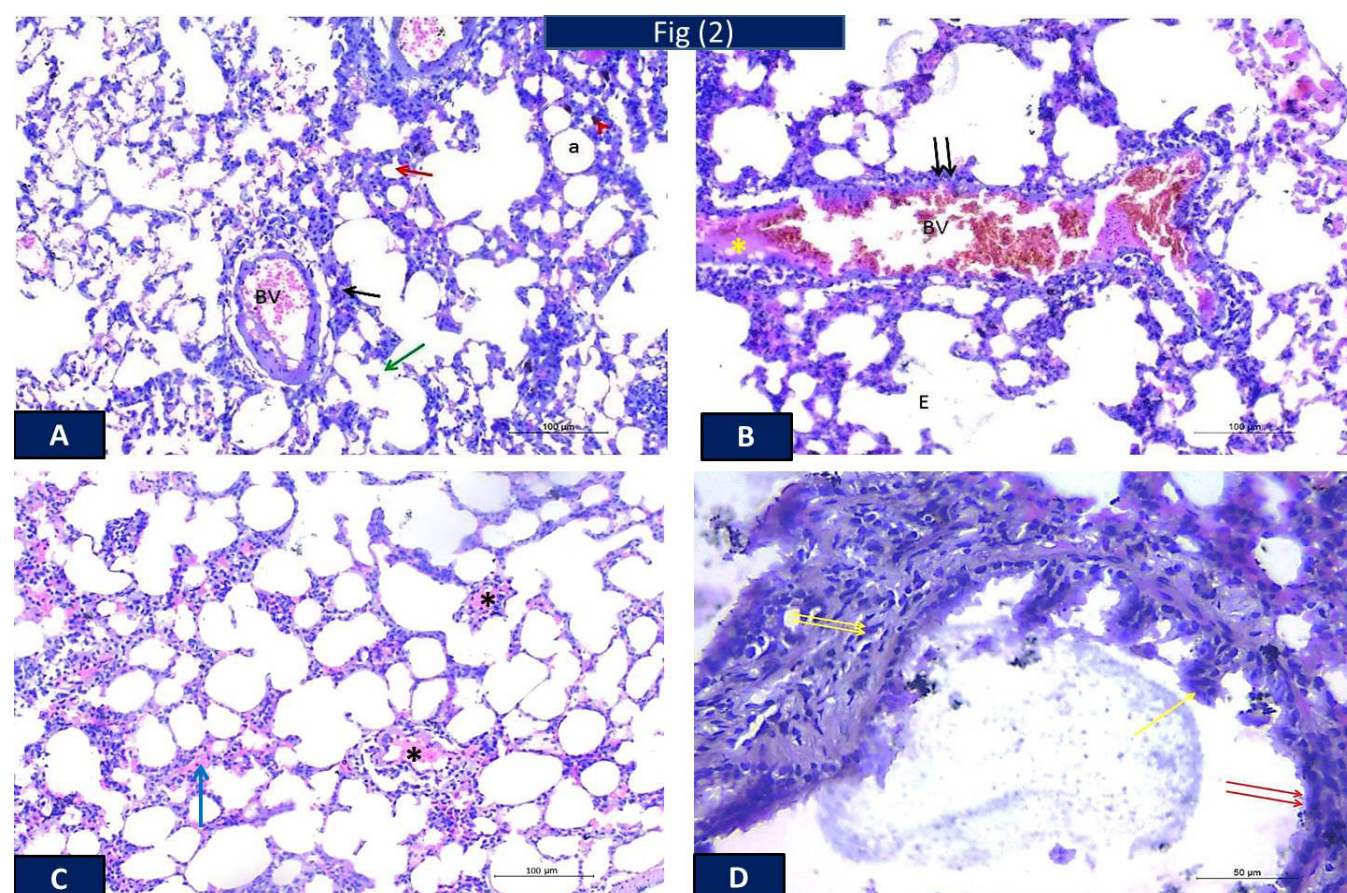


Fig 2: Photomicrographs of I/R group:- (A, B and C): showing unevenly alveolar spaces; some are compressed (red arrow) and others showing compensatory dilatation (a) with thickened and massively infiltrated inter-alveolar septa with inflammatory cells (black arrow). Destroyed septa (green arrow) are also noticed in addition to deposition of brown particles mostly hemosiderin deposition (red arrow head), congested blood vessels (BV) with perivascular inflammatory infiltration (double black arrows) and perivascular edema (yellow star). In addition, emphysema (E), intra-alveolar (black stars) and interstitial (blue arrow) edema are also observed (HxandE X 20). (D): revealing peri-bronchial inflammatory infiltrate (double yellow arrows) and focal sloughing of the bronchial epithelial cells (double red arrows) with presence of disorganized bronchial epithelial lining (yellow arrow) (HxandE X 40).

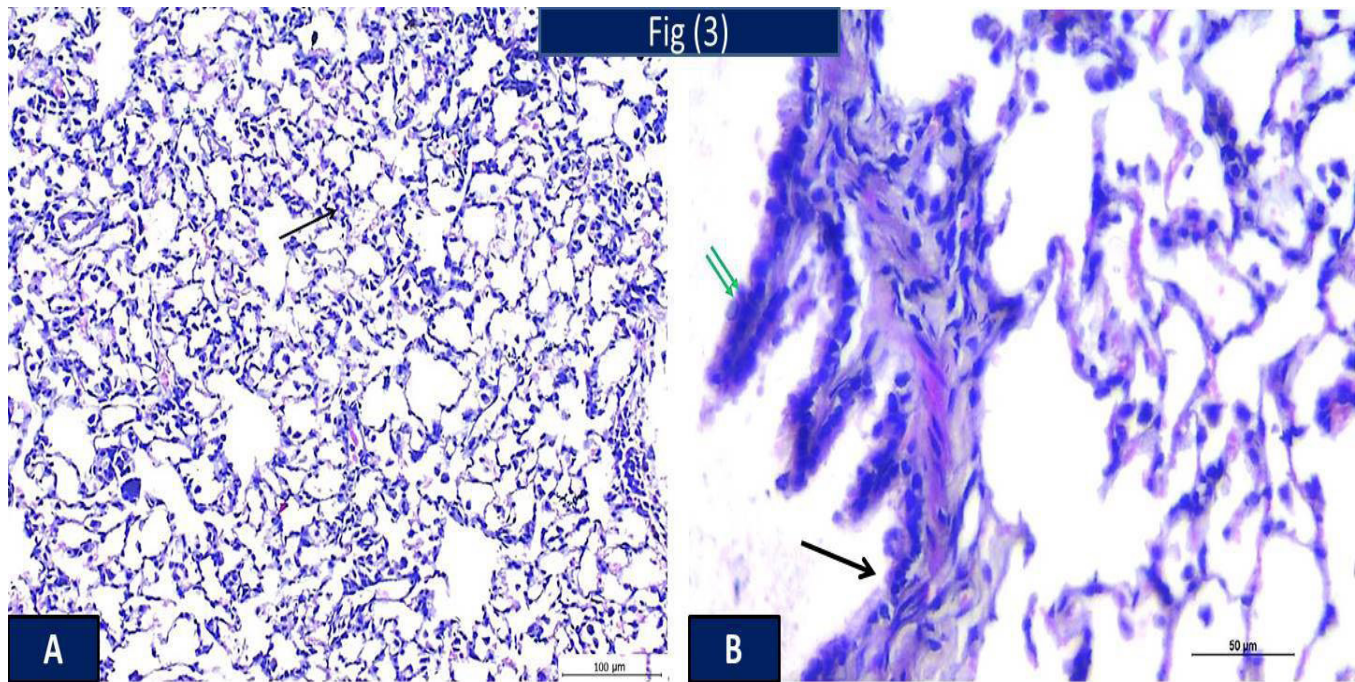


Fig 3: Photomicrographs of I/R group pretreated with pomegranate peel powder:- (A): showing more or less normal lung architecture except for some inter-alveolar inflammatory infiltrate (black arrow) (HxandE X 20). (B): revealing a bronchus lined with respiratory epithelium (double green arrows) similar to that of the control group except for sloughing (black arrow) of small area of the bronchial epithelium (HxandE X 40).

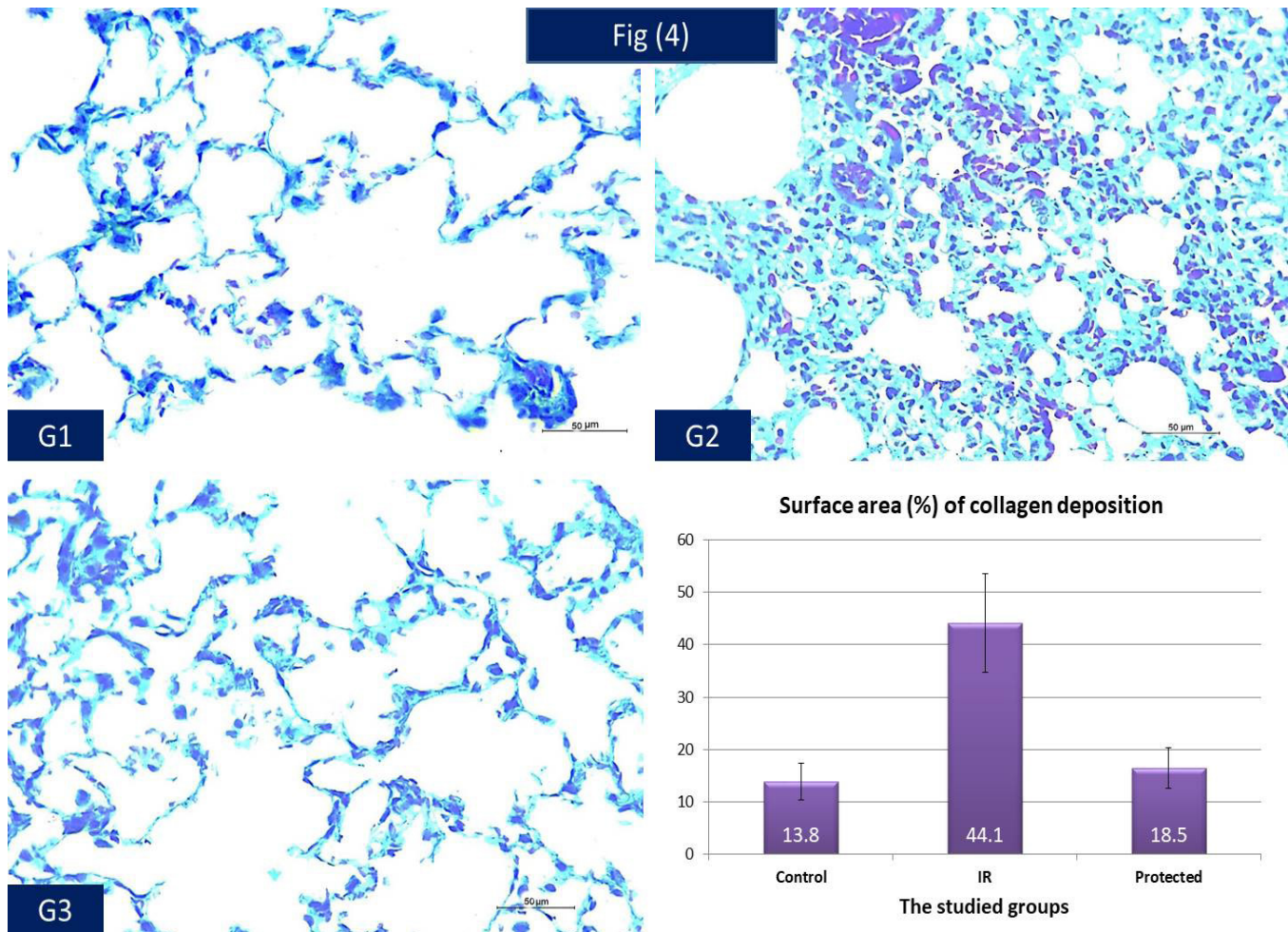


Fig 4: Photomicrographs of different experimental groups [(G1): control group, (G2): I/R group, (G3): I/R group pretreated with pomegranate peel powder] showing increase in the surface area of the collagen deposition in I/R group in comparison to the other groups (Masson Trichrome X 40).

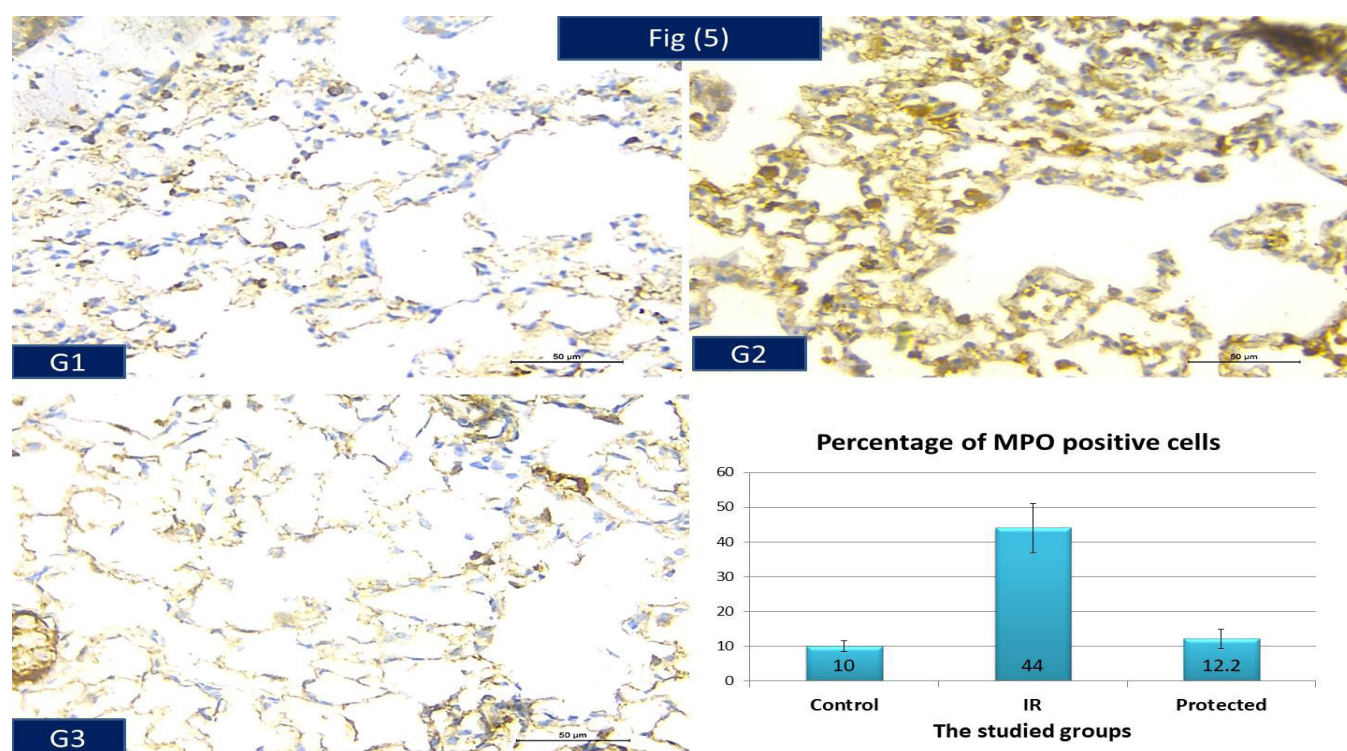


Fig 5: Photomicrographs of different experimental groups [(G1): control group, (G2): I/R group, (G3): I/R group pretreated with pomegranate peel powder] showing increase in the number of MPO positive cells in I/R group in comparison to the other groups (X 40).

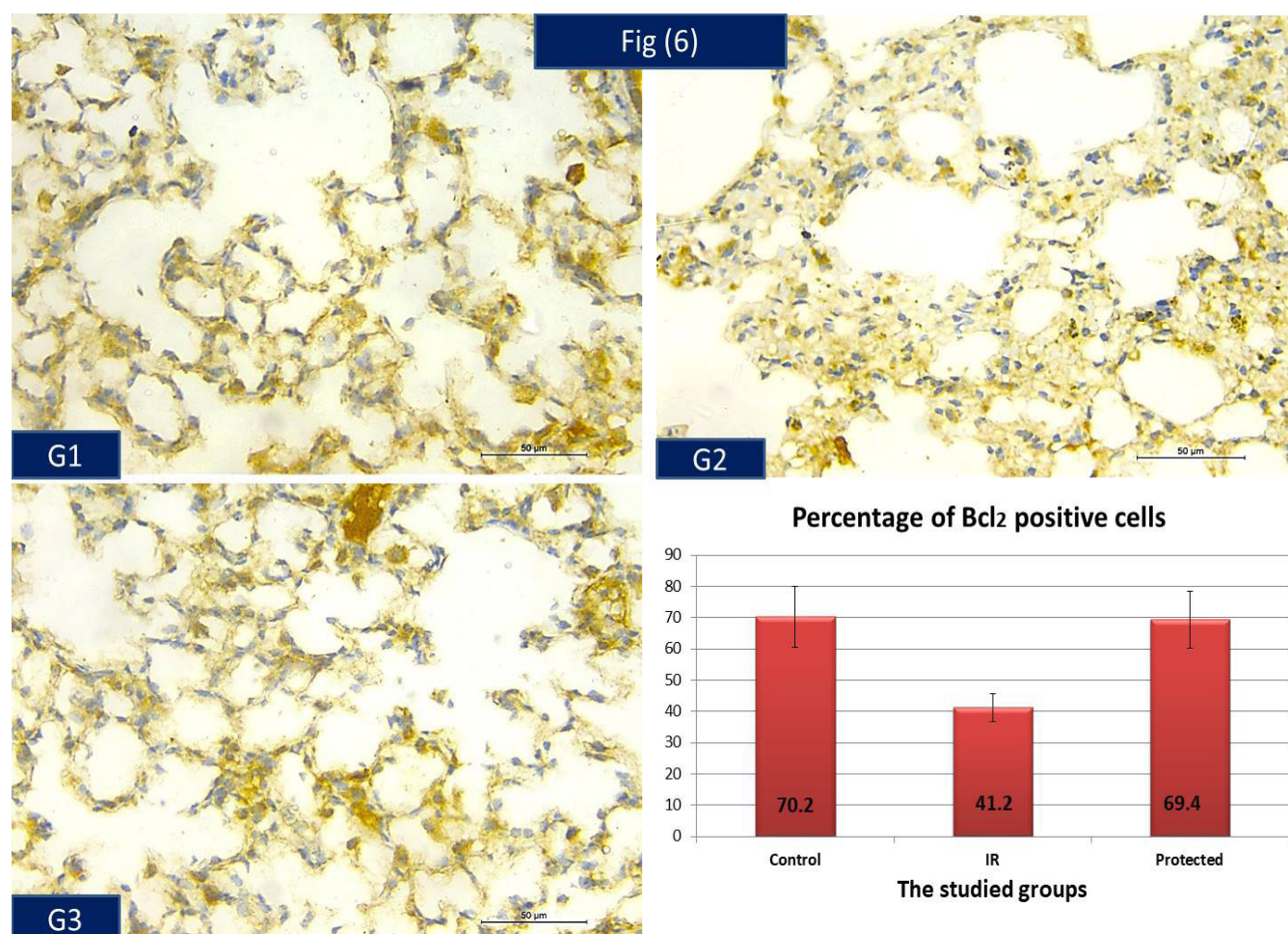


Fig 6: Photomicrographs of different experimental groups [(G1): control group, (G2): I/R group, (G3): I/R group pretreated with pomegranate peel powder] showing decrease in the number of Bcl2 positive cells in I/R group in comparison to the other groups (X40).

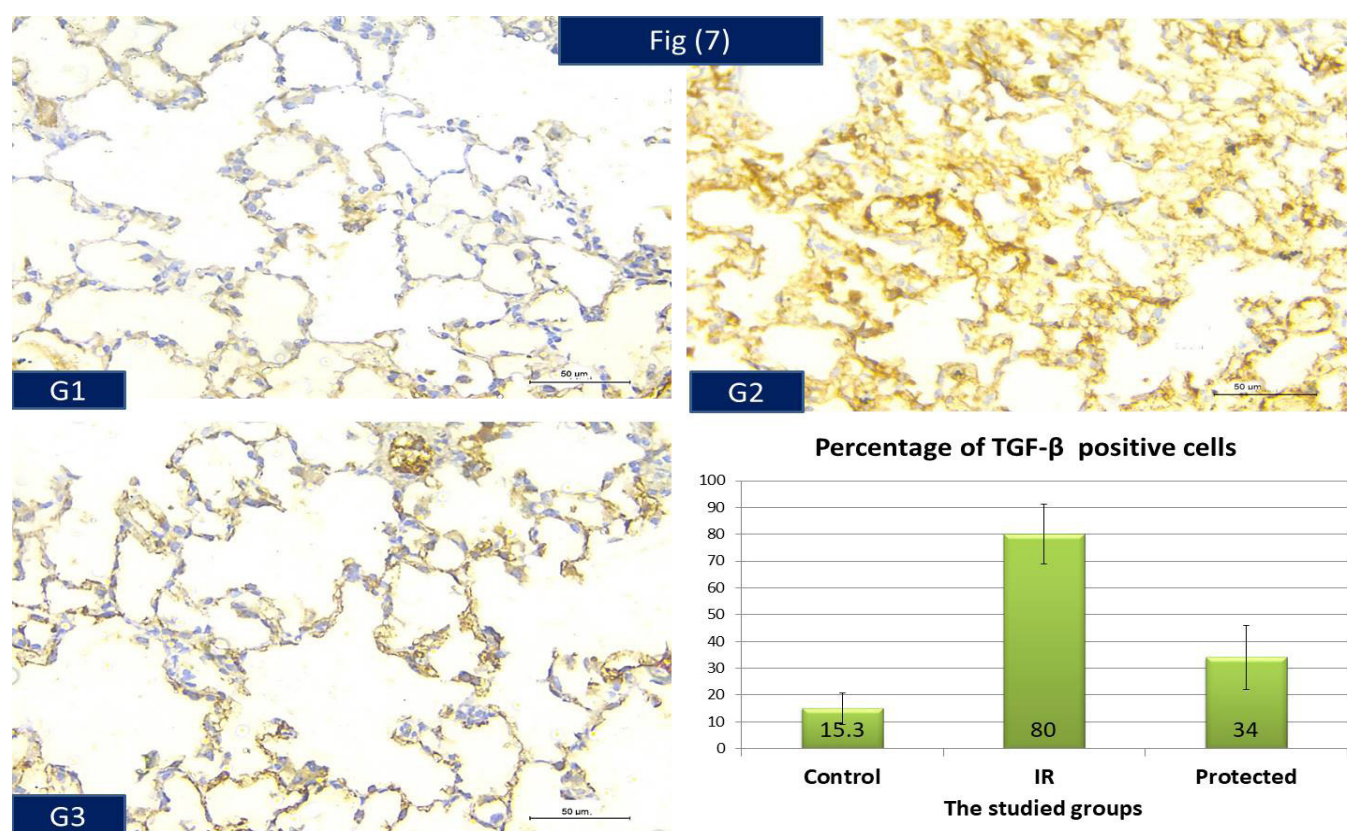


Fig 7: Photomicrographs of different experimental groups [(G1): control group, (G2): I/R group, (G3): I/R group pretreated with pomegranate peel powder] showing increase in the number of TGF-β positive cells in I/R group in comparison to the other groups (X40).

Table 1: Malondialdehyde (MDA) level among the three studied groups

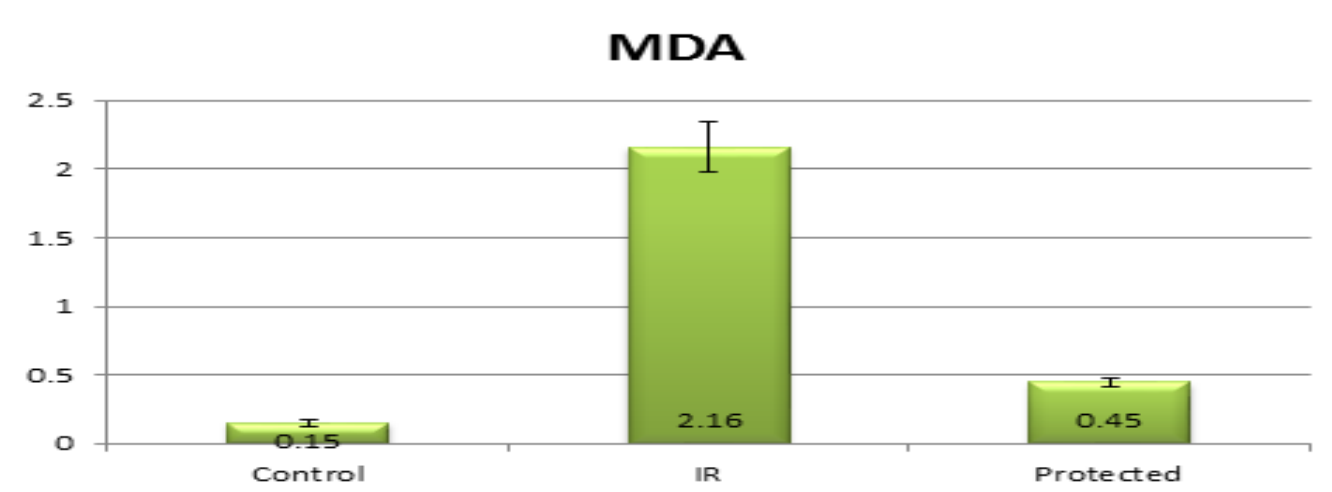
	The studied groups			U	P value
	Control	I/R	Protected		
MDA				2.69	0.008 ^a
X ±SD	0.16±0.02	2.16±0.18	0.45±0.03	2.61	0.009 ^b
Range	0.123 – 0.186	1.99 – 2.46	0.395 – 0.486	2.35	0.02 ^c

X = Mean, SD = standard deviation, U = Mann Whitney U test

a= comparing I/R group and control group

b= comparing I/R group and protected group

c= comparing control group and protected group



Histogram 1: Serum level of malondialdehyde (MDA) in control, I/R group and protected groups.

Table 2: Comparison of the three studied groups regarding the thickness of the inter-alveolar septa (in pixels):

	The studied groups			U	P value
	Control	I/R	Protected		
Thickness				2.51	0.01 ^a
X \pm SD	0.047 \pm 0.012	0.341 \pm 0.092	0.043 \pm 0.011	2.71	0.008 ^b
Range	0.03 – 0.10	0.098 – 0.508	0.01 – 0.081	0.45	0.59 ^c

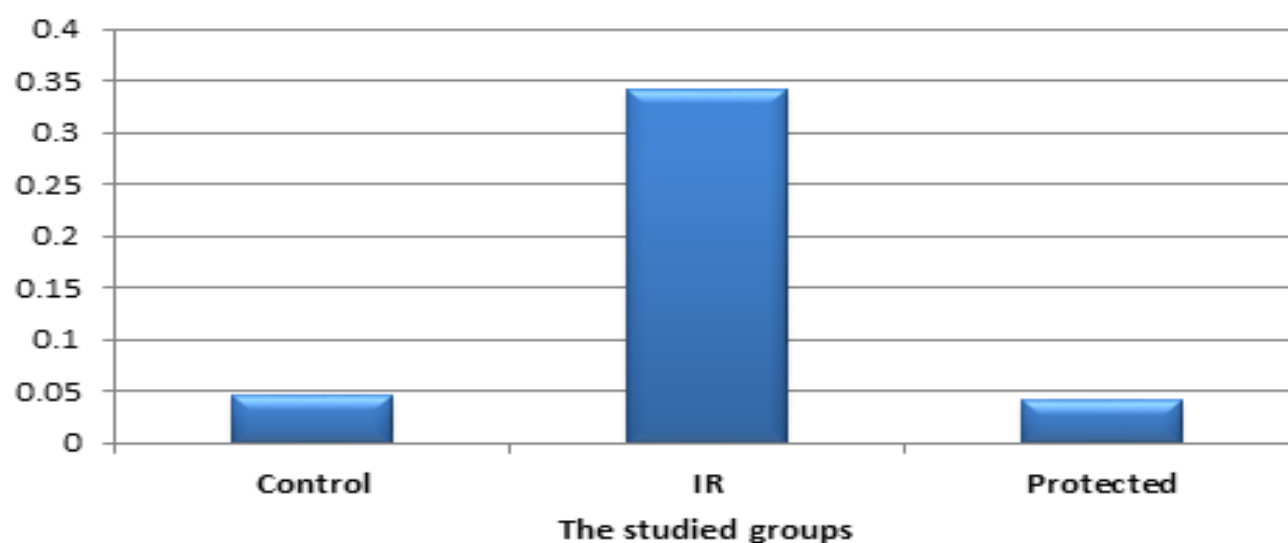
X = Mean, SD = standard deviation, U = Mann Whitney U test

a= comparing I/R group and control group

b= comparing I/R group and protected group

c= comparing control group and protected group

Thickness of interalveolar septum

**Histogram 2:** Thickness of the inter-alveolar septa in control, I/R group and protected groups.**Table 3:** Lung injury score among the three studied groups

	The studied groups			U	P value
	Control	I/R	Protected		
Score				3.91	<0.001 ^a
X \pm SD	0.6 \pm 0.12	2.60 \pm 0.52	0.80 \pm 0.18	3.98	<0.001 ^b
Range	0 – 1	2 – 3	0 – 1	0.95	0.34 ^c

a= comparing I/R group and control group

b= comparing I/R group and protected group

c= comparing control group and protected group

Table 4: Surface area (%) of collagen deposition: among the three studied groups

	The studied groups			U	P value
	Control	I/R	Protected		
Surface area (%) of collagen deposition:				3.78	<0.001 ^a
X \pm SD	13.8 \pm 3.52	44.1 \pm 9.46	18.5 \pm 4.28	3.79	<0.001 ^b
Range	9 – 19	28 – 60	12 – 25	2.31	0.02 ^c

X = Mean, SD = standard deviation, U = Mann Whitney U test

a= comparing I/R group and control group

b= comparing I/R group and protected group

c= comparing control group and protected group

Table 5: Immunohistochemistry results among the three studied groups

	The studied groups			U	P value
	Control	I/R	Protected		
MPO positive cells (%)				2.61	0.009 ^a
X ±SD	10.0±1.58	44.0±7.07	12.20±2.77	2.53	0.01 ^b
Range	8 – 12	36 – 52	9 – 15	1.27	0.21 ^c
Bcl2 positive cells (%)				2.69	0.008 ^a
X ±SD	70.2±9.73	41.2±4.49	69.4±9.18	2.41	0.02 ^b
Range	59 – 82	35 – 45	58 – 79	0.10	0.92 ^c
TGF-β (%)				2.81	0.007 ^a
X ±SD	15.3±2.77	80.0±11.18	34.0±8.22	2.61	0.009 ^b
Range	10 – 22	65 – 95	27 – 47	2.67	0.01 ^c

X = Mean, SD = standard deviation, U = Mann Whitney U test

a= comparing I/R group and control group

b= comparing I/R group and protected group

c= comparing control group and protected group

DISCUSSION:

Ischemia of the extremity is a major problem that may lead to a high incidence of morbidity and mortality. Thrombosis and embolism are the main causes of the acute limb ischemia. In addition, trauma, orthopedic surgery and free flap reconstruction frequently cause ischemia reperfusion (I/R) injury that affects various organs^[13]. Therefore, it is a major problem that threatens the life. As yet, no reports have been documented on the protective effect of pomegranate peel powder as a natural element on the damaged lung tissue as a result of skeletal muscle I/R.

The period of the ischemia and subsequent reperfusion used in this study was based on previous studies that evaluated the lesions of remote organs^[5,14,15]. The results of this research clarified that I/R caused severe lung damage in the form of vascular congestion with subsequent hemosiderin deposition, massive inflammatory infiltrate, edema, emphysema, alveolar damage with thickened inter-alveolar septa and disrupted bronchial epithelium. These results were in agreement with other researches^[14,15] that reported occurrence of acute lung injury following hind limb I/R in rats.

These changes may be attributed to generation of free radicals from reperfusion tissues that led to a state of oxidative stress affecting the lung tissue as confirmed in this study by increased level of malondialdehyde (MDA). Earlier study^[16] revealed that the reduction of oxygen supply to the tissues led to accumulation of metabolites in the cells as a result of ischemia and hence damage of vital organs post-reperfusion.

Furthermore, it was reported that during ischemia, neutrophils and macrophages produced reactive oxygen species (ROS) which in turn activated nicotinamide adenine dinucleotide phosphate (NADPH) and pro-inflammatory cytokines^[17]. The pro-inflammatory molecules could induce direct tissue damage and activate the leukocytes and thereby promote their sequestration in organs susceptible to leukocyte mediating injury such as

the lung alveolar capillary bed, leading to endothelial cell injury and increased vascular permeability as mentioned by other researchers^[18].

Moreover, it was clarified that excess ROS led to protein and DNA damage that might directly damage cellular membranes by lipid peroxidation^[19]. Malondialdehyde, an end product derived from the peroxidation of polyunsaturated fatty acids and related esters, was considered to be an indicator for lipid peroxidation as mentioned in previous study^[20]. Furthermore, other study^[5] referred the damage of the remote organs to the depletion of antioxidant capacity of the body that occurred as a result of overproduction of ROS.

Although many studies have been performed to demonstrate the systemic effect of I/R, the underlying mechanisms are not well established. In this study, significant increase in MPO and TGF-β expressions with significant decrease in the expression of Bcl2 in the lung tissue following skeletal muscle I/R was confirmed. So, we suggested that they played important roles in the pathogenesis of acute lung injury following skeletal muscle I/R. These changes may be due to the increase of ROS within the lung tissues.

This was in line with other researchers^[5] who stated that MPO was associated with an overproduction of ROS and they considered MPO as an index of accumulated activated leukocytes in the tissues that led to depletion of antioxidant capacity of the body. In addition, it was demonstrated that MPO was a local mediator of tissue damage and the resulting inflammation and hence was considered to be an important target in the treatment of inflammatory condition^[21].

In this study, the significant decrease of Bcl2, an anti-apoptotic marker, in the lung tissue of I/R group indicated a great correlation between the apoptosis-related genes and lung injury induced by skeletal muscle I/R. This could be explained by earlier studies as they suggested that mitochondria played an important role in the survival/death

of cells and that the opening of mitochondrial permeability transition pore could be regulated through reducing free radicals and regulating Bax and Bcl2 expression^[22]. They added that the oxygen free radicals directly caused structural damage of cells or opening of the mitochondrial permeability transition pore in I/R injury^[23].

In this research, the significant increase in TGF- β in the lung tissue of I/R group suggested to be one of the underlying mechanisms that reflected some of the histopathological lung changes. This was in agreement with other researchers^[24] who considered the TGF- β as a cytokine secreted by different cells as alveolar macrophage, endothelium, epithelium and fibroblast especially after multiple alveolar injuries as one of the pro-fibrotic cytokines. In addition, previous study^[25] clarified that TGF- β was a critical mediator in the development of pulmonary edema by increased alveolar permeability and it was involved in several biological processes as cell proliferation, differentiation, extracellular matrix remodeling, inflammation, apoptosis and fibrosis.

Some investigators^[26] stated that growth factors were involved in organ repair by their increased synthesis or release from the damaged cells after I/R insult. They found that there was a positive relationship between growth factors and tissue repair, and that induction of endogenous TGF- β by ischemia was necessary for tissue repair. They added that by the end of tissue repair, the level of TGF- β was restored in tissue again.

Our results revealed highly significant increase of collagen deposition in I/R group in comparison to that of control group. This may be attributed to occurrence of oxidative stress and increase of some pro-fibrotic cytokines in I/R group as confirmed in this study by the significant increase of TGF- β .

However, there are not enough documented data on increased collagen deposition in the lung tissue following skeletal muscle I/R, it was postulated that following acute inflammation, excessive ROS could induce damaged pulmonary epithelia to secrete pro-inflammatory and pro-fibrotic cytokines that could lead to imbalances between histone acetylation and deacetylation^[27]. Moreover, it was mentioned that TGF- β was the key mediator in the development of pulmonary fibrosis through its actions to induce collagen gene expression or synthesis by stimulation of fibroblast proliferation^[28,29].

It was suggested that the process of fibrosis was promoted by early pro-inflammatory mediators. So, blocking of these mediators might be one approach to attenuate fibrosis^[30].

Acute lung injury induced by hind limb I/R was significantly mitigated by pomegranate peel powder administration in this study. There was an obvious decrease in the lung tissue edema, inflammatory infiltration, alveolar damage and other features of the lung injury to a great extent as confirmed by the decrease in the histological

injury scoring. This may be contributed to the antioxidant, anti-inflammatory and anti-apoptotic and even anti-fibrotic effects of pomegranate peel powder as established in this study.

As confirmed in this research, pomegranate peel powder significantly lowered MDA level and so, improving the lipid peroxidation. Furthermore, it inhibited the cellular apoptosis in the lungs through up-regulation of Bcl2. In addition, it significantly down regulated both MPO and TGF- β in the lung tissue following I/R. Moreover, pomegranate peel powder significantly decreased the collagen deposition in the lung via the decrease in TGF- β expression in addition to its antioxidant effect.

However there are no documented researches on the protective effect of pomegranate peel powder on the lung injury induced by skeletal muscle I/R and its underlying mechanisms, some studies^[31,32,33] postulated that the pomegranate fruit and peel extract had the ability to suppress lipid peroxidation in addition to its role as an electron donor in scavenging free radicals. Furthermore, this was in agreement with Khadijeh *et al.*^[34] who investigated the antioxidant defense mechanism of pomegranate by reducing I/R induced endoplasmic reticulum stress in rat kidneys.

In conclusion, the results of our study confirmed that pomegranate peel had a protective effect against lung injury induced by I/R. These effects might be, at least in part, due to the inhibition of ROS production, down-regulation of MPO and TGF- β with up-regulation of Bcl2 in the lung tissues. So, we suggested the possibility of clinical application of pomegranate peel in I/R injury of the lung. Different dosages and alternative time protocols of pomegranate peel administration for lung injury induced by skeletal muscle I/R should be investigated in future studies.

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الملخص العربي

الدور الوقائي المحتمل لمسحوق قشر الرمان على إصابة رئة الجرذان الناتجة عن نقص التروية الدموية للعضلات الهيكلية ثم ضخها

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المقدمة: إصابة العضلات بنقص التروية الدموية ثم إعادة الضخ اثناء بعض العمليات الجراحية يؤدي الى فشل العديد من الاعضاء مما يسبب تعرض الحياه للخطر.

الهدف من البحث: دراسة الدور الوقائي المحتمل لمسحوق قشر الرمان على إصابة رئة الجرذان الناتجة عن نقص التروية الدموية للعضلات الهيكلية ثم ضخها.

الطرق والأدوات: تم تقسيم ثلاثون ذكرا من الجرذان البيضاء إلى ثلاث مجموعات (مجموعة 1) ضابطة و (مجموعة 2) معرضة لنقص التروية الدموية ثم ضخها للعضلات الهيكلية) ومجموعة 3 (تعالج بمسحوق قشر الرمان 500ملي جم/ كجم لمدة 10 ايام قبل التعرض لنقص التروية الدموية ثم ضخها للعضلات الهيكلية) فى نهاية التجربة تم سحب عينة دم لقياس المالونديالديهيد ، ثم جمع عينات من الرئة وتجهيزها لدراسة هستولوجية وهستوكيميائية مناعية.

النتائج: أظهرت جرذان المجموعة 2 إصابة حادة فى الرئة وارتشاح التهابى شديد وتورم وتدمير لبعض الحويصلات الهوائية وسماكة فى الحواجز بينهم واحتقان بالأوعية الدموية وزيادة ترسب الكولاجين و زيادة فى الميلوبيروكسيدزومحول معامل النمو بيتا ونقص فى مكافح موت الخلايا المبرمج "بي سي ال2" كما اظهرت النتائج أن استخدام مسحوق قشر الرمان أدى إلى تحسن ملحوظ فى كل هذه التغيرات.

الخلاصة: مسحوق قشر الرمان له تأثير وقائى على الرئة بعد التعرض لنقص التروية الدموية ثم ضخها للعضلات.