

Effect of Obesity on The Lung of Adult Male Albino Rat with Evaluation of The Possible Protective Role of Pomegranate : Light and Electron Microscope Study

Original
Article

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ABSTRACT

Background: Pomegranate is rich in bioactive compounds, mainly polyphenols and anthocyanins, with known health benefits, due to its high antioxidant capacity.

Aim of the work: Is to study the protective effects of pomegranate on the histological changes induced by obesity on the lung of the adult male albino rat.

Materials and methods: The present study was carried on 40 adult male albino rats divided into 4 equal groups: control group kept on standard diet for 3 months, group II (kept on standard diet and pomegranate juice in a dose 20ml/Kg orally for 3 months), group III (received high fat diet for 3months), and group IV (received both high fat diet and pomegranate juice at the same previous doses and period). At the appropriate time, the specimens were taken and prepared for light and electron microscopic study.

Results: Light microscope examination revealed structural alterations in group III in the form of inflammatory cellular infiltration with desquamated epithelial cells inside the lumens of bronchioles and alveoli. Collapsed alveoli with fragmented elastic fibers in their wall and intra-alveolar cellular infiltration were detected. Congested blood vessels with increased lipid droplets inside pneumocyte type II were also noticed. Increased peri-bronchiolar, peri-vascular and inter-alveolar collagen deposition with interrupted elastic lamina in bronchioles and around alveoli were also detected. Ultrastructural examination of specimens taken from group III showed increased lipid droplets compressing the nuclei with disorganized multilamellar bodies in type II pneumocytes. Neutrophils and eosinophils were seen inside the alveoli. Amelioration of the previous histological changes was detected in group IV after weight reduction.

Conclusion: it was concluded that pomegranate has anti-obesity effects which ameliorated the previous histological changes induced in the lung by receiving high fat diet.

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Key Words: Lung, obesity, pomegranate.

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INTRODUCTION

Obesity is one of the most common and important health concerns facing our society today^[1]. An increase in the prevalence of obesity in almost all countries in the world has led the World Health Organization to define it as "The global epidemic"^[2,3]. The more severe the obesity is, the more serious the medical complications and mortality risks are; hence, the term morbid. Morbid obesity affects 3 to 5 million Americans, just over half of whom are women^[4,5]. Childhood obesity affects the developed and developing countries^[6]. Obese children are susceptible to the development of chronic diseases such as type 2 diabetes and cardiovascular disease^[7].

Obesity has recently been identified as a major risk factor for development of asthma which tends to be more severe in obese individuals. It does not also respond

adequately to treatment in those individuals. As a result, the combination of obesity and asthma is becoming a major public health issue in many countries^[8]. However, one of the most recent studies, stated that, approximately two thirds of the patients suffering from chronic obstructive lung diseases is overweight or obese^[9]. Clinical experience also indicates that the combined restrictive/obstructive deficits evident in obese patients with COPD culminate in worsening symptomatology and activity limitation^[10].

It was stated that weight loss in obese persons of any age can decrease the obesity-related medical complications and increase physical function and quality of life^[11]. There are several risks associated with the pharmacologic and surgical interventions of obesity, suggesting that a dietary intervention may be the safest and most cost-effective option for those who are moderately obese^[12-15]. There are many dietary strategies that have been shown to affect

energy balance in a manner that results in successful weight loss^[16, 17 and 18]. The use of synthetic antioxidants in food has been decreased due to their suspected action as promoters of carcinogenesis, as well for the general consumer rejection of synthetic food additives. The pomegranate is one of the important dietary sources of antioxidant phenolics. Extracts from pomegranate have been used since ancient times to treat many conditions such as parasitic infections, ulcers, diarrhea, dysentery, hemorrhage, microbial infections, and respiratory pathologies. Extracts from the different parts of this plant have been researched for numerous pharmacologic activities, such as antitumor, antibacterial, astringent, antidiarrheal, and anti-obesity activities^[19, 20 and 21].

This research was carried out to study the histological changes of induced obesity on the lung of adult male albino rat and evaluate the role of pomegranate in ameliorating these changes.

MATERIALS AND METHODS

Experimental Design

This study was conducted on 40 male albino rats weighing 120±20gm. They were housed in clean properly ventilated cages under the same environmental conditions with free access to standard laboratory diet and water throughout the experiment. The composition of the standard diet used in this study included: 23.5% proteins, 48.8% carbohydrates, 5% lipids, 12% water, 5% ash, 5% cellulose, and a 0.7% mixture of vitamins and minerals. The diet was designed at Tanta Company for Oils and Soap, Gharbyia, Egypt. Animals were allowed a two weeks pre-experimentation period to be acclimatized to the laboratory conditions. All animals received humane care in accordance with the guidelines of research ethics committee, Tanta Faculty of Medicine, Egypt.

Pomegranate juice was obtained by squeezing using a commercial blender (Braun blender, Germany) and was filtered to remove the residue. The juice was used within 1 hour after squeezing and filtration^[22].

High fat diet was prepared by adding 20% animal (lamp) fat + 1% cholesterol to the standard diet^[23]. Cholesterol was purchased from Sigma Company, Egypt which is in the form of white powder in plastic bottles each containing 100 grams. The high fat diet was prepared every two days, kept at 4°C until used and left at the room temperature one hour before use.

The animals were divided into four main groups (10 animals each)

Group I

It served as a control group which was kept on standard rodent chow diet for 3 months.

Group II

Animals of this group were kept on standard diet and

were given pomegranate juice orally by gastric tube at a dose of 20 ml/kg body weight daily for 3 months^[24].

Group III

The animals of this group were kept on high fat diet for 3 months.

Group IV

The animals of this group were kept on high fat diet at the same composition in group II for 3 months^[25] concomitant with administration of pomegranate at the same dose in group II.

After 3 months, weight of groups I and II were 150±20 gm, group III was 220-250 gm but group IV reached 145-180 gm after 3 months.

At the appropriate time, the animals were sacrificed under ether anesthesia. Thoracotomy was performed and the lungs were dissected and perfused by intratracheal inflation with a fixative solution (2% paraformaldehyde and 2% glutaraldehyde solution in 0.1 M phosphate buffer pH 7.2). The trachea was ligated just caudal to the larynx and the thoracic contents were removed en block as one unit, then both lungs were excised, processed for light and electron microscopic study. For light microscopy, specimens of the right lung were immersed in 10% neutral-buffered formalin for 24 hours, washed, dehydrated, cleared and embedded in paraffin. Then, 5 µm sections were stained with hematoxylin & eosin (H&E), Mallory's triple stain for demonstration of collagen fibers and Orcein stain (for demonstration of elastic fibers)^[26].

For electron microscopy, small pieces of the left lung were fixed at 4°C in 2.5 % glutaraldehyde in 0.1 M phosphate buffered saline (pH 7.3) for two hours, rinsed in 0.1M phosphate buffered saline and post fixed in phosphate-buffered 1% osmium tetroxide for one hour, then dehydrated in ascending grades of ethanol. After immersion in propylene oxide, the specimens were embedded in epoxy resin mixture. Semithin sections (1µm thick) were stained with 1% toluidine blue and examined by light microscope for proper orientation. Ultrathin sections (80-90nm) were stained with uranyl acetate and lead citrate^[27] to be examined by Joel electron microscope (Akishima, Tokyo, Japan) in the EM unit, Faculty of Medicine, Tanta University.

Statistical analysis

All data (initial weight, final weight of rats, BWG percentage, and collagen fibers area percentage) for all groups were expressed as mean ± SD. Statistical analyses were carried out using SPSS software (SPSS Science, version 11.0.1, Chicago, Illinois, USA). ANOVA and Post-Hoc test were used to compare between groups. $P \leq 0.05$ and 0.001 were considered significant and highly significant, respectively. For collagen fibers area percentage, the image analyzer system Leica Qwin 500 (Solms, Germany) in the Central Research Laboratory in Tanta University College of Medicine was used. Mallory trichrome stained sections

at 400 X magnification were examined and the positive data were calculated.

RESULTS

1) Body weight

The results indicated that there was no significant difference in initial body weight between all experimental groups (Table 1). Concerning final body weight and body weight gain percentage (BWG %), the data showed that pomegranate received group (group 2) revealed a non-significant change in the final body weight and BWG % as compared to control group (group 1). The high fat diet group (group 3) showed a statistically significant increase in both final body weight and BWG % as compared to control group. Concerning group (4), it was found that there was no significant change in the final body weight and BWG % as compared to control group and it revealed significant decrease when compared with high fat diet group (Table 2, 3).

2) Light microscopic results

Light microscopic examination of H&E stained sections from group I and II revealed normal lung in which the spongy structure of the lung appeared with thin inter-alveolar septa with normal clear alveoli (Fig. 1 a). Group III showed inter-alveolar inflammatory cell infiltration. Some alveoli were collapsed. Blood vessels were congested. (Fig 1 b and c). All these changes were improved after weight reduction in group IV (Fig 1d).

Mallory's triple stained sections obtained from group I and II showed normal amount and distribution of collagen fibers in the walls of the bronchiolar passages. Scanty delicate collagen fibers were seen in the inter-alveolar septa (Fig 2a). Group III showed increased peri-bronchiolar, peri-vascular and inter-alveolar collagen deposition (Fig 2b). Group IV showed moderate amount of collagen deposition in peribronchiolar, perivascular and in interalveolar septa (Fig 2c).

Examination of Orcein stained sections obtained from group I and II revealed continuous elastic fibers, in lamina propria of bronchiole, internal and external elastic

lamina of vessels and interalveolar septa (Fig.2d). Group III showed interrupted or discontinuous elastic lamina in bronchioles (Fig.2 e). Group IV showed regain of the normal configuration of the elastic fibers, however, there is some interruption around the bronchioles which is less than that seen in group III (Fig.2f).

2) Morphometric results

Statistically significant increase in the area percentage of Mallory trichrome stained collagen fibers was shown in group III (high fat diet group) in comparison to group I (standard diet) and II (standard diet + Pomegranate). Group IV (high fat diet + Pomegranate) showed statistically significant decrease compared to group III. There was no statistically significant difference between group VI and group I and II. Group I and II did not show any statistically significant difference. (Graph 1 and Table 4).

3) Electron microscopic results

Transmission electron microscopic examination of specimens obtained from animals of control group I and II revealed that most of the alveolar surface was covered by type I pneumocytes, each with a single nucleus showing condensed peripheral chromatin and surrounded by a perinuclear region of cytoplasm. Type II pneumocytes appeared as simple cuboidal cells at the corners of the alveoli. The cytoplasm contained small membrane bound secretory vesicles that contain concentric lamellae surrounded by a unit membrane (Fig. 3a).

In group III, type I pneumocytes were hardly seen. However, type II pneumocytes showed increased lipid droplets compressing their nuclei with disorganized multilamellar bodies. Neutrophil and eosinophil were seen inside the alveoli. Macrophages with their processes engulfing the nearby cellular debris, fibroblasts with excess collagen beside and congested blood vessels were also observed (Fig.3.b, c, d and e). Improvement in the electron microscopic picture of the lung parenchyma with increase in phagocytic cells as blood monocyte and alveolar macrophage could be seen in group IV (Fig.3 f).

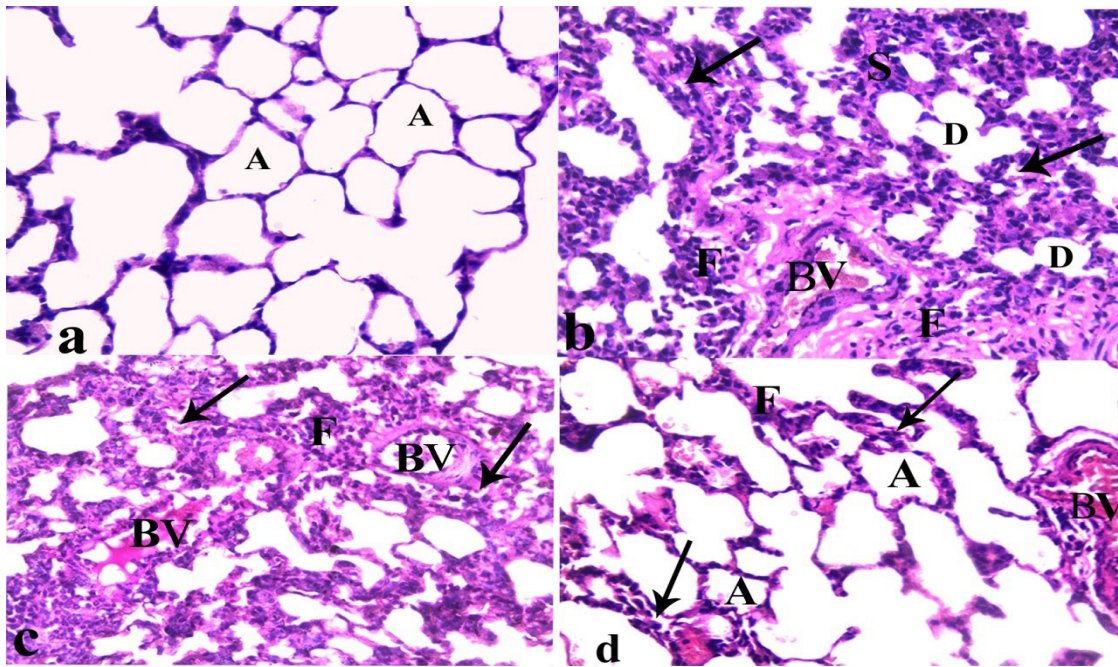


Fig. 1: Light photomicrographs of lung parenchyma of albino rats, a: Rats fed with a standard chow diet, showing normal alveolar spaces (A) with thin inter-alveolar septa around. b & c: Animals receiving high-fat diet showing collapsed alveoli (arrows) separated by thick inter-alveolar septa (S), compensatory dilatation of other alveoli (D), inflammatory cellular infiltration (F), congested blood vessels (BV). d: Rats after weight reduction showing many normal thin walled alveoli (A), few collapsed alveoli (arrows), with areas of mild cellular infiltration (F) and few congested blood vessels (BV), (H & E), Mic, Mag X 400

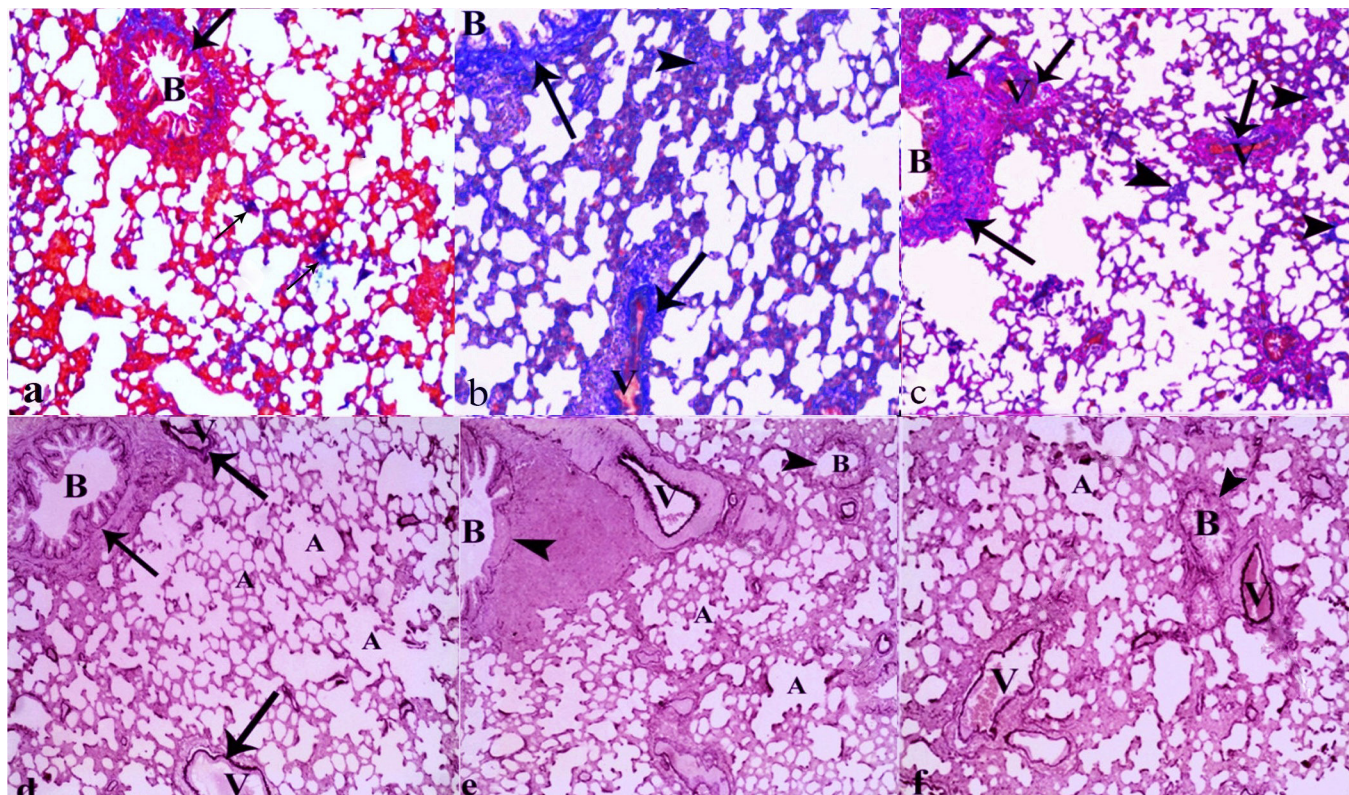


Fig. 2: Photomicrographs of lung parenchyma of albino rats, a: Rats fed with a standard chow diet, showing normal bronchiole with minimal collagen fibers around (blue color, arrow) and in inter-alveolar septa (thin arrows). b: Animals receiving high-fat diet showing excess amount of collagen deposition in peribronchiolar, perivascular areas (arrows) and in thickened interalveolar septa (arrowheads). c: Rats after weight reduction showing moderate amount of collagen deposition in peribronchiolar, perivascular (arrow) and in interalveolar septa (arrowheads). (Mallory Trichrome stain, Mic, Mag X 200). Photomicrographs of Orcein stained sections showing: d: continuous elastic fibers (dark brown color, arrows), prominent in control group in lamina propria of bronchiole, internal & external elastic lamina of vessels. e: interrupted or discontinuous elastic fibers in bronchioles (arrow heads) in animals receiving high-fat diet. f: regained normal elastic fibers after weight reduction with some interruption around bronchioles which is less than that seen in animals receiving high fat diet. (Orcein stain X200) B: bronchiole, V: blood vessel, A: alveoli.

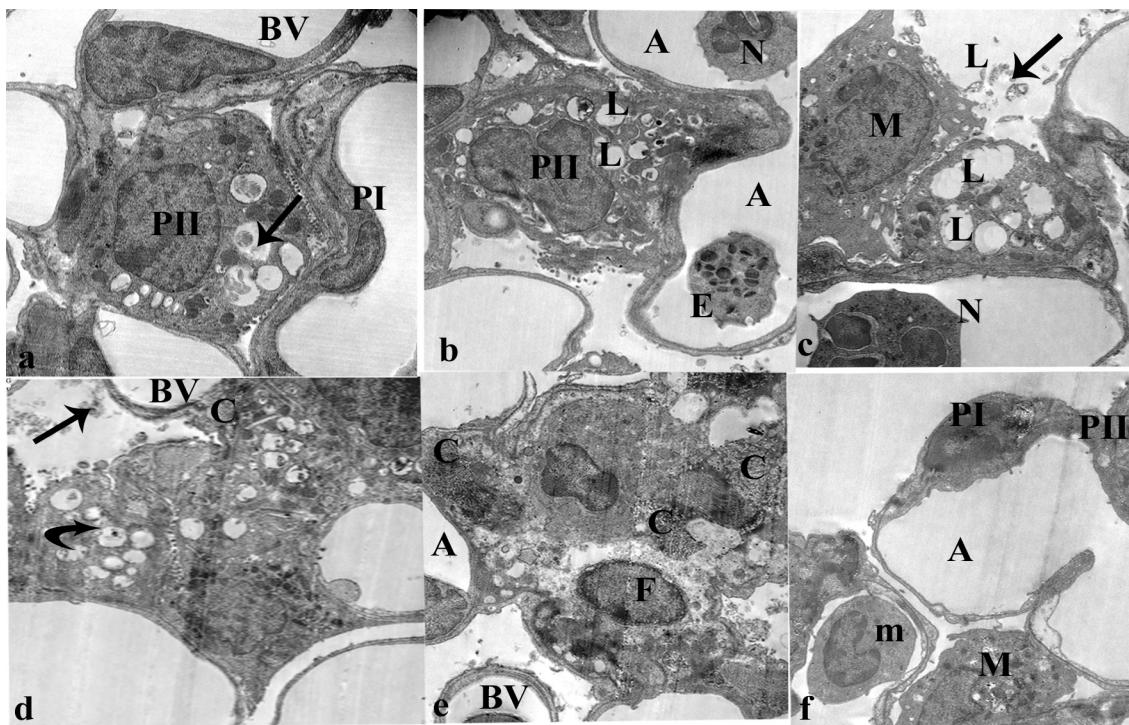
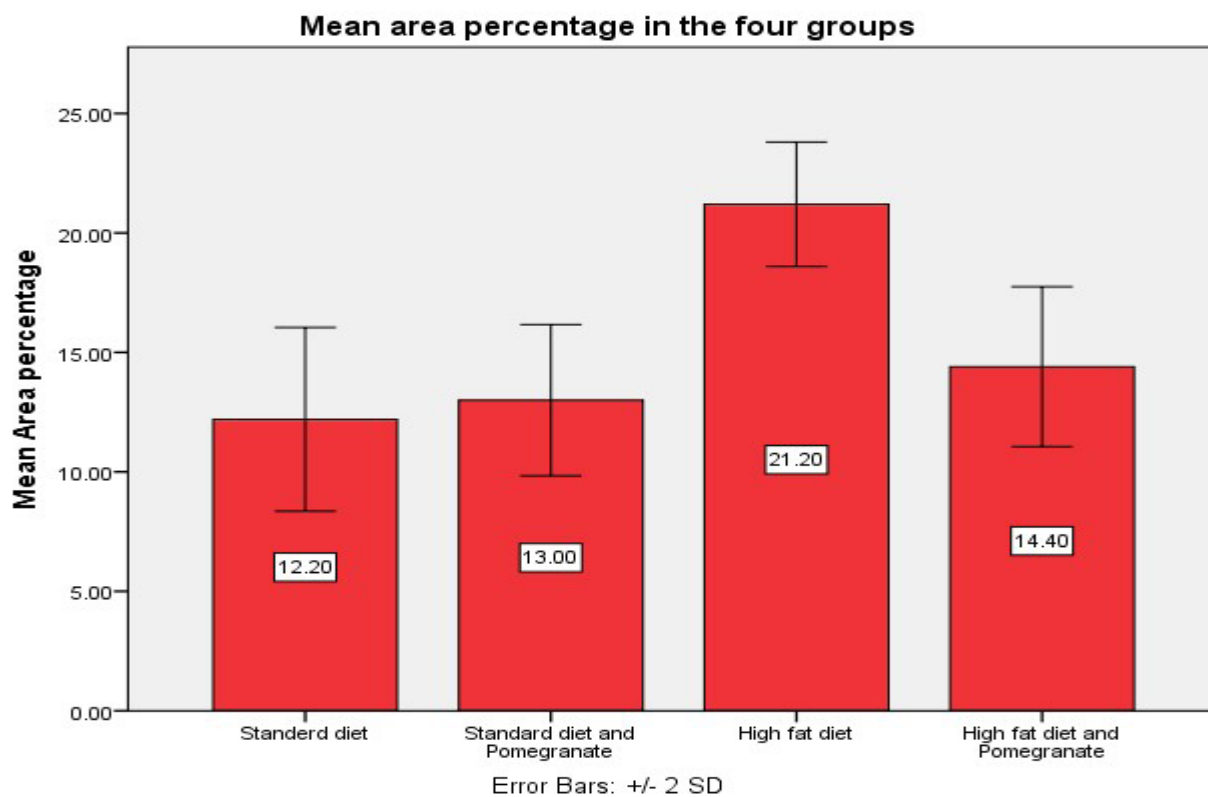


Fig. 3: Electron microscopy of lung parenchyma showing normal blood vessels (BV), pneumocyte type I with its flat nucleus & pneumocyte type II with its rounded nucleus and well organized multilamellar bodies (arrow) in control rats (fig.a). Specimens from obese rats (figs. b, c, d and e) showing neutrophil (N) and eosinophil (E) inside the alveoli (A) with increased lipid droplets in type II pneumocytes compressing their nuclei (L) with disorganized multilamellar bodies (curved arrow). Note, Macrophages (M) with their processes engulfing the nearby cellular debris(arrow), fibroblasts (F) with excess collagen besides(C) and congested blood vessels. Rats after weight reduction show improvement in the electron microscopic picture of the lung parenchyma with increase in phagocytic cells as blood monocyte (m) and alveolar macrophage(M).
 PI: pneumocyte type I PII: pneumocyte type II



Graph 1: Mean area percentage for collagen fiber deposition the four groups.

Table 1: Comparison between groups as regard mean ± SD of the initial body weight:

Initial weight	Standard Diet (group1)	Standard Diet and Pomegranate (group 2)	High Fat Diet (group 3)	High fat diet and Pomegranate (group 4)
Mean ± SD	159.29 ± 14.84	162.86 ± 13.80	167.86 ± 9.94	166.43 ± 14.06
<i>F value</i>			0.757	
<i>P value</i>			0.561	
1 & 2	1 & 3	1 & 4		3 & 4
0.617	0.234	0.320		0.841

Table 2: Comparison between groups as regard mean ± SD of the final body weight:

Final weight	Standard Diet (group1)	Standard Diet and Pomegranate (group 2)	High Fat Diet (group 3)	High fat diet and Pomegranate (group 4)
Mean ± SD	182.14 ± 12.86	182.86 ± 12.86	220.71 ± 13.05	195.71 ± 10.97
<i>F value</i>			12.635	
<i>P value</i>			0.001*	
1 & 2	1 & 3	1 & 4		3 & 4
0.912	0.001*	0.068		0.001*

*: Highly significant.

Table 3: Comparison between groups as regard mean ± SD of the body weight gain %:

Body weight gain %	Standard Diet (group1)	Standard Diet and Pomegranate (group 2)	High Fat Diet (group 3)	High fat diet and Pomegranate (group 4)
Mean ± SD	14.58 ± 4.05	12.41 ± 2.49	31.50 ± 1.64	17.85 ± 3.96
<i>F value</i>			34.176	
<i>P value</i>			0.001*	
1 & 2	1 & 3	1 & 4		3 & 4
0.237	0.001*	0.078		0.001*

*: Highly significant.

Table 4: Comparison between Mean Area Percentage for Standard Diet, Standard Diet + Pomegranate, High Fat Diet, and High fat diet + Pomegranate for collagen fiber deposition

	Standard Diet (group1)	Standard Diet and Pomegranate (group 2)	High Fat Diet (group 3)	High fat diet and Pomegranate (group 4)	<i>P- value</i>
Mean Area Percentage	12.2±1.9	13 ±1.5	21±1.3	14.4±1.7	<i>P</i> = 0.9 <i>P</i> 1<0.001* <i>P</i> 2<0.03** <i>P</i> 3<0.3*** <i>P</i> 4=1**** <i>P</i> 5<0.001*****

P: Between groups 1 and 2

*: Between groups 1 and 3

** : Between groups 2 and 3

*** Between groups 1 and 4

**** Between groups 2 and 4

***** Between groups 3 and 4

Comparison was done using ANOVA and Post Hoc test

DISCUSSION

Obesity is an independent risk factor for many medical conditions such as diabetes, hypertension, coronary heart disease, high cholesterol levels, depression, musculoskeletal problems, and several cancers. Risks are also higher for some non-fatal conditions such as back pain, arthritis, infertility, and, in many Western countries, poor psychosocial functioning^[2]. It occurred as a result of an energy imbalance, where energy intake is greater than energy consumption. The prevention and treatment of this energy imbalance requires modifications not only in lifestyle in terms of physical fitness but also an increased intake of natural foods that help increase energy expenditure by stimulating thermogenesis and fat oxidation^[28,29].

The present study revealed different changes in specimens taken from rats on high fat diet in the form of increased peri-bronchiolar collagen deposition and inflammatory cellular infiltration especially, fibroblasts and macrophages. Some alveoli were collapsed with intra-alveolar inflammatory cell infiltration especially, neutrophils and eosinophils. Blood vessels were congested and there were increased lipid droplets in the cytoplasm of pneumocytes type II. All these changes were improved after weight reduction.

This goes in line with *Saraiva et al.*^[30] who found that experimentally induced obesity in mice led to alveolar collapse, broncho-constriction, increased collagen fiber content in airways and alveolar septa. Also, the high fat diet led to a further increase in epithelial cell detachment, eosinophil and neutrophil infiltration, and elastic fibers fragmentation. They explained that obesity yielded larger chest wall circumferences, which resulted in lower tidal volume, alveolar collapse, and a reduction in the diameter of airways.

In this line, Shore and colleagues^[31] have shown that obese mice have increased airway hyper-responsiveness independent of lung volume, possibly associated with augmentation of the inflammatory process. The impact of obesity on the remodeling process may result from chronic repetitive injury to the airway wall caused by inflammation^[32]. Increased IL-4 and IL-5 may play a role in the regulation of collagen synthesis by stimulating transforming growth factors (TGF) and increasing the expression of type I collagen through activation of transcription factors^[33]. Furthermore, VEGF may also cause a marked increase in inflammation, followed by infiltration with mononuclear cells, eosinophils, and neutrophils in lung parenchyma^[34] as was proved in this study. Also, *Lumeng et al.*,^[35] and *Foster D J et al.*,^[36] concluded that obesity is accompanied with pulmonary inflammation and remodeling due to lipid droplet accumulation in lungs through pulmonary lipotoxicity. In agreement with the present study, *Ghobashy et al.*^[37] found that collagen deposition and elastic fiber destruction could be reversed when inflammatory process improved due to removal of the triggering cause, as air pollution in their study and obesity in the present study.

It was also stated that obesity is a low-grade chronic inflammatory condition. It has been associated with adipocyte-derived inflammatory mediators; among these, interleukin-6 (IL-6) has a primary effect on metabolism through several mechanisms. IL-6 affects adipose tissue-specific gene expression, triacylglycerol release, lipoprotein lipase downregulation, and insulin sensitivity^[38]. Many studies in humans have demonstrated that obesity is associated with an inflammatory state that in turn induces oxidative stress^[39,40]. An exposure to increased levels of reactive oxygen species has been shown to facilitate adipocyte differentiation *in vitro*^[39,41].

Previous studies explained that, feeding of rats with high fat diet leads to an increase in cholesterol absorption and hence an increase in serum cholesterol and triglycerides^[42]. Hyperlipidemia may be also due to a decrease in catecholamine level which leads to low β_2 - adrenergic receptor function and decrease lipolysis that help in decreasing fat catabolism and increasing the circulating lipid levels^[43].

In the present work, it was observed that daily administration of pomegranate juice ameliorated the previous changes. These results agree with findings of other researchers^[44]. In this regard, it was reported that pomegranate juice had a protective effect against carbon tetrachloride induced hepatic damage in rats^[45], this effect could be attributed to the radical scavenging antioxidant constituents. The principal antioxidant polyphenols in pomegranate juice include the ellagitannins and anthocyanins which have been shown to be the antioxidant responsible for scavenging the free radicals^[46].

In addition, antioxidant effect of flavanoids found in pomegranate enhanced the process of regeneration, this might be due to destruction of free radicals, supplying a competitive substrate for unsaturated lipids in the membrane and/or accelerating the repair mechanism of damaged cell membrane^[47]. The antioxidant activity of pomegranate components has been the subject of many studies^[48]. Furthermore, pomegranate extract has also been shown to preserve the antioxidant enzymes catalase (CAT), Glutathione Peroxidase (GPx) and Superoxide Dismutase (SOD) from the effects of toxic chemicals^[49,50]. In addition, the ability of pomegranate extract to protect DNA and preventing chromosomal damage in mice was proved^[51]. Another study revealed that pomegranate flower activated peroxisome proliferator-activated receptor that decreased circulated lipids, cardiac tissue triglyceride content and plasma total cholesterol^[52]. It was also stated that constituents from the pomegranate have been described to decrease IL-6 production and thus decrease symptoms of obesity^[53].

Lei et al.^[54] demonstrated the dual anti-obesity effects of pomegranate extract: through inhibition of lipase activity and the suppression of energy intake. It has been reported that the effect of the pomegranate extract on energy intake is done through decreasing hyperlipidemia by inhibiting

pancreatic lipase activity in vitro and increasing fecal fat excretion. The pomegranate extract has been observed to markedly decrease the calorie intake of mice fed an HFD but not of those fed a normal diet. The mechanism by which the appetite in obese mice decreased is not known and needs further research.

Similar findings have been demonstrated in different animal models. For example, *McFarlin et al.*^[55] reported that the consumption of pomegranate seed oil and an HFD resulted in decreases of body weight, leptin, insulin, and increased adiponectin compared with controls. *Fu et al.* reported the association of a low plasma level of adiponectin with the development of obesity, insulin resistance, and cardiovascular disease. The decrease in body weight was assumed to be mediated by the leptin–adiponectin pathway because leptin and adiponectin are closely related to body weight and composition^[56,57].

In this research, the treatment with pomegranate caused a detectable decrease of the collagen fiber content deposition in the lung, these effects could be related to its antioxidant, antifibrotic and antiapoptotic properties^[58]. These findings agree with many studies in recent years that demonstrated a correlation between the role of pomegranate peel extract and juice in regulating vital cellular functions, including cell proliferation and differentiation and its potent antioxidant activity and free radical scavenging capability^[59].

CONCLUSION

From the previous study, it was concluded that Pomegranate is a safe food that can be beneficial to the human body. The routine supplementation of PJ or its extracts may prevent or even correct obesity. Different studies have demonstrated the safety of pomegranate dietary supplements in rats, but there have not been many studies evaluating their safety in humans.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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تقييم الدور الوقائي للرمان لتأثير السمنة على رئة ذكر الجرذ الأبيض البالغ دراسة باستخدام الميكروسكوب الضوئي والإلكتروني

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المقدمة: الرمان غني بالمركبات النشطة بيولوجيا وخاصة البولي فينولات و الأنثوسيانينات المعروفة بفوائدها الصحية وقدرتها العالية كمضادات للاكسدة.

الهدف من البحث: هو دراسة التأثير الوقائي للرمان على التغيرات الهستولوجية التي أحدثتها السمنة على رئة ذكر الجرذ الأبيض.

مواد و طرق البحث: أجريت الدراسة الحالية على أربعين من ذكور جرذان التجارب البيضاء البالغة و التي تم تقسيمها الى اربعة مجموعات متساوية: المجموعة الأولى (الضابطة) تناولت نظام غذائي قياسي لمدة ثلاثة شهور، المجموعة الثانية تناولت أيضا نظام غذائي قياسي مع اعطاء عصير الرمان بجرعة 20مل لكل كيلو جرام من وزن الجسم يوميا لمدة ثلاثة شهور، المجموعة الثالثة تناولت غذاء عالي المحتوي الدهني لمدة ثلاثة شهور، أما المجموعة الرابعة فقد تناولت في نفس الوقت عصير الرمان و غذاء عالي المحتوى الدهني بنفس الجرعات السابقة لمدة ثلاثة شهور. وبعد انقضاء الفترة المحددة تم تخدير الحيوانات وأخذ عينات الأنسجة من الرئة ومعالجتها للفحص بالمجهر الضوئي والإلكتروني.

النتائج: أظهر الفحص بالمجهر الضوئي وجود تغيرات في التركيب الهيكلي لجرذان المجموعة الثالثة منها وجود خلايا التهابية و حويصلات هوائية منهارة واحتقان في الأوعية الدموية. وقد لوحظ أيضا وجود زيادة في ترسب ألياف الكولاجين حول القصيبات و الأوعية الدموية وبين الحويصلات الهوائية و صفيحة مطاطية متقطعة في القصيبات. وعند الفحص بالمجهر الإلكتروني للخلايا الرئوية النوع الثاني تبين وجود زيادة في القطرات الدهنية بداخلها لدرجة تضغط على النواة مع وجود أجسام غير منتظمة متعددة الطبقات ووجود خلايا النيوتروفيل و الإزينوفايل بداخل الحويصلات الهوائية. كما أظهرت الدراسة باستخدام المجهر الضوئي والإلكتروني وجود تحسن جزئي في التغيرات السابقة في التركيب الهستولوجي بالمجموعة الرابعة بعد تخفيض الوزن.

الاستنتاج: نستخلص من هذه الدراسة أن الرمان له تأثير وقائي ضد السمنة ويعمل على إنقاص الوزن وبذلك فهو يقلل من التغيرات الهستولوجية التي أحدثتها الأغذية عالية المواد الدهنية على الرئة.