COMPARATIVE STUDY OF PROTECTIVE EFFECT OF OLIVE OIL WITH OR WITHOUT GENTAMICIN ON *E COLI*-INDUCED LUNG SEPSIS IN ADULT MALE ALBINO RAT

ABORAYA, A. and ALI, A. M.

1Department of Histology, Faculty of Medicine, Tanta University, Egypt; 2Department of Medical Physiology, Faculty of Medicine, Fayoum University, Egypt. E. mail: aaboraya3@gmail.com, Cell: +201120332233.

Received: December 26, 2016; Accepted: January 12, 2017

Abstract: Sepsis is frequently the cause of severe pulmonary dysfunction. Gentamicin is used clinically due to its wide spectrum of activities against infections; however it has serious adverse effects and life-threatening toxicity. The challenge for scientists is to develop alternative therapy or protective agents against its toxicity. Hence the present study is an attempt to elucidate the possible protective role of virgin olive oil with or without gentamicin against the toxic changes on the lung induced by sepsis. Thirty six adult male albino rats were used and divided into three groups. Group I: served as the control group, Group II: lung sepsis was induced by intraperitoneal injection of *E. coli* LPS serotype O127 : B8 and Group III: lung sepsis was induced as in group II then divided randomly into 3 subgroups. Subgroup IIIa: animals were injected with gentamicin, Subgroup IIIb: animals were injected with gentamicin and given virgin olive oil and Subgroup IIIc: animals were given virgin olive oil only. During the experiment the body weight and hemodynamic parameters were monitored. At the end of the experiment the lungs were excised out and processed. Histological and immunohistochemical studies of lung sepsis showed an alteration in the lung architecture in the form of overexpansion of alveoli alternating with collapse of others. Pneumocytes II showed destruction of lamellar bodies and were predominant replacing pneumocytes type I in the alveolar lining with increase in HSP70 expression, significant decrease in body weight and blood pressure with tachycardia. Gentamicin-treated group revealed minimal improvement in lung tissue. While concomitant administration of virgin olive oil along with gentamicin showed a noticeable improvement in lung architecture and increase in HSP70 expression with significant improvement of body weight and blood pressure. However animals given virgin olive oil only revealed the most favorable results with remarkable improvement of lung architecture. In conclusion, based on the previous findings, gentamicin and olive oil prevents the deleterious effects of lung sepsis. Moreover, virgin olive oil solely has succeeded to improve the septic effects on lung by enhancing anti-oxidant defense system and suppression of oxidative stress.

Key words: Olive oil, Gentamicin, Lung sepsis, HSP70

INTRODUCTION

Sepsis is the most common cause of death among critically ill patients and is associated with a systemic inflammatory response that affects several organs and resulting in organ failure. Elderly are more vulnerable to be affected with development of lung injury coupled with sever pulmonary
dysfunction, which increases the rate of morbidity and mortality [1,2].

Acute inflammatory reaction arises as a response to induction of sepsis by infection resulting in a rapid and profound increase of lung vascular permeability with activation and recruitment of neutrophils to the lung. Activation of circulating granulocytes is characterized by increased production of both pro- and anti-inflammatory mediators. Increased expression of pro-inflammatory cytokines such as tumor necrosis factor (TNF-α), interleukin (IL)-6, and anti-inflammatory cytokines such as (IL)-10 in the circulation [3,4]. In addition to increased production of reactive oxygen metabolites which are strongly associated with death [4,5].

On the other hand, the heat shock response is a highly conserved endogenous cellular mechanism that protects against injury and environmental stresses including infection, oxidative damage, hypoxia, and thermal stress. HSP70 expression is one of the Heat Shock Response and belong to the best characterized proteins in lung biology [6,7]. Induction of the heat shock response may improve outcome from pathophysiological disturbances. Thus, HSP70 may protect against subsequent exposure to severe stresses possibly through attenuation of pro-inflammatory and inhibits NF-kB activation or cyclooxygenase-2-regulated pathways and also altering cytokine expression [8].

Gentamicin (aminoglycoside antibiotic) has been used in treatment of various infectious diseases. It is widely used clinically in sepsis due to its wide spectrum of activities against Gram negative bacterial infections caused by Pseudomonas, Proteus and Serratia [9]. It is considered the only effective therapeutic drug against bacterial strains resistant to other antibiotics. However, serious toxicity is a major limitation to its use. The most notable toxic effect is highly obvious on the kidney and inner ear, yet other organs are also involved [9,10].

Gentamicin induces cellular injury and necrosis via several mechanisms, including increased generation of reactive oxygen species (ROS) and reduced efficiency of antioxidant enzymes in the tissues. Besides, suppression of reticuloendothelial system and synthesis of nucleic acids (DNA, RNA) as well as liposomal disturbance. Although the potential nephrotoxic effects and broad side effects of prolonged aminoglycoside therapy, gentamicin still continues playing a useful role in treatment due to its effectiveness [11,12]. Many researches were conducted to study some of the natural products including plants, herbs, and certain foods containing antimicrobial substances for their antimicrobial activity [12,13].

Olive oil is recognized for its natural composition of monounsaturated fatty acids which unlike animal fats. It is also known to have high levels of antioxidants with beneficial role for health. The positive effects of virgin olive oil have been attributed to the content of polyphenols, phenolic compounds, oleic acid and tocopherols, which exert antioxidant, anti-cancer, antiviral, anti-atherogenic, anti-inflammatory, antimicrobial, hypoglycemic, hepatic, cardiac and neuro-protective effects [14,15]. Other researchers added that an enteral nutrition containing olive oil benefits the survival of mice during sepsis [16].

The increased incidence of sepsis with high sepsis mortality rates, complex pathophysiology and overall difficulties in treatment, made sepsis and sepsis-associated multi-organ failure a challenge for scientists and clinicians. Therefore, the aim of this research was to evaluate the protective role of virgin olive oil with or without gentamicin during \textit{Escherichia coli}-induced lung sepsis.

**MATERIALS AND METHODS**

**Animals:** In this work, thirty six adult male albino rats weighing between 150 and 200 g each obtained from animal center in King Faisal University. All rats were placed in clean properly ventilated cages and were fed the ordinary laboratory diet with adequate water supply and allowed to acclimate for 3–4 days on a 12:12-h light-dark cycle. The experimental protocol was approved by the local Animal Care Committee of King Faisal University. The experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals. The animals were divided into three main groups.

**Experimental design:** Group I (control group) (n=12): Animals were equally subdivided into two subgroups. Subgroup (Ia): received no treatment and
were used to study the normal histological structure of the lung and as the base line of study as regards blood pressure and heart rate. Subgroup (Ib): each animal was injected with 0.5 mL phosphate-buffered saline intraperitoneal (ip.) once daily for 12 days and given virgin olive oil (sigma chemical company) at a dose of 2ml /kg body weight (b.wt.) once daily [17] orally by stainless steel feeding needle for 12 days.

**Group II (induced-lung sepsis) (n=6):** Lung sepsis was induced in these animals by intraperitoneal injection with *Escherichia coli* LPS serotype O127:B8 (10 mg/kg b. wt. diluted in 0.5 ml of phosphate-buffered saline) [18]. The organism was obtained from the Microbiology and Infectious Diseases Department in Collage of Medicine, King Faisal University.

**Group III (treated-group) (n=18):** Lung sepsis was induced in all animals by the same procedure of group II and all treatments were started one day after *E. coli*–injection [18]. The animals divided randomly into three subgroups of six animals each.

Subgroup (IIIA): animals were injected (ip.) with gentamicin (Memphis Company for Pharmaceutical and Chemical Industries, Cairo, Egypt) (100 mg/kg b.wt.) once daily for 12 days [19]. Subgroup (IIb) animals were injected (ip.) with gentamicin (100 mg/kg b.wt.) once daily and given virgin olive oil at a dose of (2ml /kg b.wt.) orally once daily for 12 days. Subgroup (IIlc) animals were given only virgin olive oil at a dose of (2ml /kg b.wt.) orally once daily for 12 days.

**Body weight:** The weight of the animals was measured at the beginning and at the end of the experiment (day 0 and day 12). The body weight was measured with the aid of a weighing balance and the percentage of change of body weight was calculated.

**Hemodynamic monitoring:** The heart rate and blood pressure were recorded daily two hours after treatment, throughout the time of experiment to evaluate the hemodynamic effects of olive oil with or without gentamicin in induced- lung sepsis. The new (Harvard Apparatus Advanced Blood Pressure Monitor) used a non-invasive cuff to measure blood pressure indirectly based upon the Korotkoff method. The animal was allowed to enter the holder freely and remained in the holder at least 10 to 15 minutes before pressure measurements begin, an occlusion tail-cuff placed on the animal’s tail to occlude the blood flow. Upon deflation automatic calculation of systolic, diastolic and mean arterial blood pressure with continuous heart rate monitor. At least four consecutive measurements were recorded and the average was calculated. One day following the end of the experiment (after 12 days), the animals were anesthetized with ip. injection of thiopental sodium at a dose of 40 mg/kg [20] and sacrificed. The lungs were rapidly dissected out, washed immediately with saline and processed for light, electron microscopy and immunohistochemical study.

**Immunohistochemical study:** Paraffin sections of the lung were deparaffinized, rehydrated, incubated in hydrogen peroxide for 10-15 minutes, washed in buffer citrate and stained with uranyl acetate and lead citrate [22] and examined using a JEM 1011 (JOEL, Tokyo, Japan) electron microscope at 80 KV in Faculty of Medicine, Department of Biomedical sciences, King Faisal University, Kingdom of Saudi Arabia.
night at 4°C in a humidified chamber. On the next day, all slides were washed in buffered phosphate solution, incubated with biotinylated anti-mouse antibody diluted 1:200 for 30 minutes at room temperature. Then, they were washed and incubated in streptavidin peroxidase (Thermo Scientific, Lab Vision Corporation-Fremont, CA, USA) for ten minutes at room temperature. Positive cells were stained in brown color was developed with 0.05% 3, 3’-diaminobenzidine tetrachloride (DAB) solution (Thermo Scientific, Lab Vision Corporation-Fremont, CA, USA) as chromogen peroxidase-compatible. Finally, the slides were counterstained with Meyer’s hematoxylin. The specificity of the immune reactions was tested by replacing the primary antiserum with phosphate buffer saline as a negative control [23].

Morphometric study: Images were obtained using an Olympus light microscope (BX50, Tokyo, Japan) coupled to an Olympus digital camera (C-7070, Tokyo, Japan). Image analysis was performed using software “Image J” (National Institute of Health, Bethesda, Maryland, USA). Lung specimens from each group were examined for the mean color intensity of positive immunohistochemical reaction for HSP-70 (in anti-HSP70 mouse monoclonal antibodies -stained sections): 10 different non-overlapping randomly selected fields at a magnification of 400 were examined in each slide. The color intensity was calculated by subtracting the color intensity of negative immunohistochemical control from the color intensity of positively stained specimens in serial sections.

Statistical analysis: Body weight, hemodynamic parameters as well as the data obtained from the image analyzer was collected and coded to facilitate data manipulation and double entered into Microsoft Access. The data were analyzed using one-way analysis of variance (ANOVA) test followed by Tukey’s procedure for comparison between the groups using the statistical package for the social sciences software (SPSS Inc., Chicago, Illinois, USA). The values were expressed as mean ± SD and percentages. P value ≤ 0.05 was considered significant [24].

RESULTS

Light microscopic results: Control group I (both Subgroups: Ia and Ib): revealed no histological difference between both of subgroups. Control sections showed normal spongy structure and architecture of the lung. The sections showed bronchioles lined with simple columnar partially ciliated epithelium and surrounded by continuous smooth muscle layer, alveolar sacs, alveoli with clear alveolar cavities, thin interalveolar septa and normal blood vessels (Fig.1). The lining epithelium of alveoli was composed of type I pneumocytes and type II pneumocytes. Type I pneumocytes were squamous cells with flattened nuclei that covered most of the alveolar surface. Type II pneumocytes were few, cuboidal cells having large round nuclei. Alveolar macrophages were found bulging from the interalveolar walls (Fig.2).

Group II (induced-lung sepsis): Lung sections showed marked histological alteration of the lung tissue. Overexpansion of many alveoli with destruction of the interalveolar septa and connection of the alveoli together as well as collapse of many alveoli and thickening of the interalveolar septa were all observed. The wall of bronchiole appeared partially attenuated or exofoliated with partial desquamation of their lining epithelium and interrupted muscle layer (Fig. 3). The alveoli appeared mostly lined by cuboidal cells (type II pneumocytes) while type I pneumocytes appeared to be few or even lost. Many alveolar macrophages are found bulging in the alveolar lumen and on the surface of alveoli (Fig. 4). Blood vessels were dilated and interstitial hemorrhage was observed. Focal areas were severely affected with loss of normal lung architecture and heavily infiltration with mononuclear inflammatory cells mainly around bronchioles and in the interalveolar septa (Figs. 3,4).

Group III (treated-group): Subgroup IIIa (animals treated with gentamicin): Lung sections showed preserved alteration of lung tissue where, focal areas of over expansion of alveoli and collapse of others with thickening of interalveolar septa. Bronchioles showed intact wall with partially exofoliated and desquamated lining epithelium, however the muscle layer was intact and surrounded the wall of the bronchioles (Fig. 5). Alveoli were mainly lined by cuboidal cells (type II pneumocytes) projecting into the lumen of the alveoli while type I pneumocytes were found to be scanty. Multinucleated
macrophages were observed bulging on the surface and in the lumen of alveoli (Fig. 6). Dilated and congested blood vessels (Fig. 5) and interstitial hemorrhage were observed. Furthermore, the lung tissue was heavily infiltrated with mononuclear inflammatory cells (Figs. 5, 6).

Subgroup IIIb (animals treated with gentamicin and virgin olive oil): Lung sections showed focal areas of collapsed alveoli separated by thickened interalveolar septa and the wall of bronchioles lined by simple columnar partially ciliated epithelium (Fig. 7). Focal areas appeared infiltrated with mononuclear inflammatory cells almost around bronchioles and interalveolar septa (Figs. 7, 8). However, other normal alveoli showed reappearance of type I pneumocytes which appeared more frequent as well as type II pneumocytes were appeared less frequent as compared with group IIIa. In addition, the alveolar macrophages were bulging from the interalveolar septa (Fig. 8).

Subgroup IIIc (animals treated with virgin olive oil only): lung sections showed nearly normal histological picture of lung architecture as compared with control group (Fig. 9). The alveoli appeared patent with thin interalveolar septa and normal blood vessels. The lining epithelium of the alveoli was composed mainly of squamous cells (type I pneumocytes) and few large cuboidal cells (type II pneumocytes) with alveolar macrophages bulging from the surface of alveoli. However, focal areas of mild interstitial mononuclear cellular infiltration and hemorrhage were still observed (Fig. 10).

**Transmission electron microscopic results:**

**Control group I** (both subgroups: Ia and Ib): Ultrathin sections of lung specimens showed the two types of pneumocytes lining the alveoli. Type I pneumocytes were flattened in shape with a flat large nucleus filling a large part of the cytoplasm and have smooth surface (Figs. 11, 12). Type II pneumocytes were rounded cells with round euchromatic nuclei and few short scattered microvilli on their cell surfaces. Their cytoplasm contained numerous mitochondria and numerous lamellated bodies with concentric or parallel lamellae distributed on one side of the nucleus (Fig. 11). The alveolar macrophages were observed as large cells in the alveolar lumen with large irregular nuclei and their cytoplasm contained few organelles and lysosomes. Some pseudopodia were seen (Fig. 12).

**Group II (Induced-lung sepsis):** Ultrathin sections of lung specimens, showed few type I pneumocytes

---

**Explanation of figures**

**Fig (1):** A photomicrograph of a lung section from group I (control) showing normal spongy structure of lung, bronchioles (B) lined with partially ciliated simple columnar epithelium (thin arrows) and surrounded by muscle layer (M), alveolar sacs (As) and alveoli (A) with normal alveolar spaces and thin interalveolar septa (S). Normal blood vessels (V) are seen. (H&E X 200)

**Fig (2):** A photomicrograph of a lung section from group I (control) showing lining epithelium of alveoli, composed of squamous cells (type I pneumocytes) (thin arrows) and large cuboidal cells (type II pneumocytes) (thick arrows). Notice the alveolar macrophages (arrow heads) bulging from the interalveolar walls. (H&E X 400)

**Fig (3):** A photomicrograph of a lung section from group II (induced-lung sepsis) showing bronchiule (B) with desquamated lining epithelium (asterisk) and interrupted muscle fibers (M) in the bronchiolar wall with inflammatory cells (F). Areas of alveolar expansion (AE) and collapse of others alveoli (A) with thickened interalveolar septa (S). Intersitial hemorrhage (h) is seen. (H&E X 200)

**Fig (4):** A photomicrograph of a lung section from group II (induced-lung sepsis) showing marked loss of architecture and collapse of the alveoli (A) with thickened interalveolar septa (S). Notice the lining of alveoli with many cuboidal cells (type II pneumocytes) (thick arrows) and few type I pneumocytes (thin arrows). Many alveolar macrophages (arrow heads) in the alveolar spaces and pronounced mononuclear infiltrating cells (F) with interstitial hemorrhage (h) are seen. (H&E X 400)

**Fig (5):** A photomicrograph of a lung section from subgroup IIIa (gentamicin-treated) showing bronchiule (B) lined with partially ciliated simple columnar epithelium, few desquamated epithelial cells on the lumen (asterisk) and surrounded by continuous muscle layer (M). Collapse of many alveoli (A) with thick interalveolar septa (S) and expansion of other alveoli (AE) are seen. Notice dilated and congested blood vessels (V), perivascular and interstitional mononuclear cellular infiltration (F) and interstitial hemorrhage (h). (H&E X 200)

**Fig (6):** A photomicrograph of a lung section from subgroup IIIa (gentamicin-treated) showing focal areas of alveolar expansion (AE), collapse of many alveoli (A) and lined with many cuboidal cells (type II pneumocytes) (thick arrows) and scanty type I pneumocytes (Thin arrows). Notice multinucleated alveolar macrophages (arrow heads) and interstitial mononuclear cellular infiltration (F) with interstitial hemorrhage (h). (H&E X 400)
Histogram 1: Morphometric analysis of the HSP70 immunohistochemical reaction in lung specimens of all groups. Data are expressed as mean ± standard deviation, *P < 0.05 is significant.
**Table (1):** Morphometric analysis of the HSP70 immunohistochemical reaction in lung specimens of all groups. Data are expressed as mean ± standard deviation, *P < 0.05 is significant.

<table>
<thead>
<tr>
<th>Group I</th>
<th>Group II</th>
<th>Subgroup IIIa</th>
<th>Subgroup IIIb</th>
<th>Subgroup IIIc</th>
<th>ANOVA P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.45±2.13</td>
<td>16.48±5.27*</td>
<td>15.47±3.46*</td>
<td>10.96±3.12*</td>
<td>7.82±1.84</td>
<td>0.0013</td>
</tr>
</tbody>
</table>

**Table (2):** Showed data of body weight and percentage of change of the body weight before and after the experiment. Data are expressed as mean ± standard deviation + P-value <0.05 is significant (Comparisons was made between body weight before and after treatment in each group). *P-value <0.05 is significant (Comparisons was made between parentage of change of body weight in each group with control group).

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Body weight before treatment</th>
<th>Body weight after treatment</th>
<th>% of change of body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>176±15.7</td>
<td>180.3±16.7</td>
<td>0.024 ±0.015</td>
</tr>
<tr>
<td>Group II</td>
<td>173.7±16.7</td>
<td>169.9±16.1</td>
<td>-0.017±0.006*</td>
</tr>
<tr>
<td>Subgroup IIIa</td>
<td>177.2±16.1</td>
<td>173.9±16.2</td>
<td>-0.019±0.007*</td>
</tr>
<tr>
<td>Subgroup IIIb</td>
<td>176.4±15.8</td>
<td>173.8±16.1</td>
<td>-0.015±0.012*</td>
</tr>
<tr>
<td>Subgroup IIIc</td>
<td>172.9±12.6</td>
<td>172±12.9</td>
<td>-0.017±0.006*</td>
</tr>
</tbody>
</table>

**Table (3):** Showed the hemodynamic parameters and heart rate. Data are expressed as mean ± standard deviation. *P-value <0.05 is significant (Comparisons was made between each group with control group). #P-value <0.05 is significant (Comparisons was made between each group with lung sepsis group II). SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial blood pressure, HR: heart rate

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group I</th>
<th>Group II</th>
<th>Subgroup IIIa</th>
<th>Subgroup IIIb</th>
<th>Subgroup IIIc</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP Mean</td>
<td>133.8±8.3</td>
<td>97.5±6.8*</td>
<td>97.8±5.6*</td>
<td>109.3±10.8*</td>
<td>106.8±6.9*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP Mean</td>
<td>97.5±5.7</td>
<td>64±5*</td>
<td>64.5±4.7*</td>
<td>74.7±6.3*</td>
<td>70.6±5.5*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MAP Mean</td>
<td>111.3±4.7</td>
<td>73.2±7.6*</td>
<td>75.6±4.7*</td>
<td>86.2±7.3*</td>
<td>82.7±5.7*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HR Mean</td>
<td>357.3±35.3</td>
<td>424.8±15.8*</td>
<td>376.1±25</td>
<td>362.1±38.3*</td>
<td>366.5±35*</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Explanation of figures**

**Fig (7):** A photomicrograph of a lung section from subgroup IIIb (gentamicin and virgin olive oil-treated) showing alveolar sacs, alveoli (A) with normal alveolar spaces, thin interalveolar septa and focal areas of collapsed alveoli (asterisk) surrounded by thick interalveolar septa (S). Bronchioles (B) lined with partially ciliated simple columnar epithelium (thin arrows) are seen. Notice focal areas of peri-bronchiolar and interalveolar mononuclear cellular infiltration (F). (H&E X 200)

**Fig (8):** A photomicrograph of a lung section from subgroup IIIb (gentamicin and virgin olive oil-treated) showing alveoli lined with type I pneumocytes (thin arrows) and type II pneumocytes (thick arrows). Notice some collapsed alveoli (asterisk) with mild thickness of interalveolar septa (S), alveolar macrophages (arrow heads) and interstitial mononuclear cellular infiltration (F). (H&E X 400)

**Fig (9):** A photomicrograph of a lung section from subgroup IIIc (virgin olive oil-treated) showing bronchioles (B) lined with partially ciliated simple columnar epithelium (thin arrow) and surrounded by intact muscle layer (M), alveolar sacs (As) and alveoli (A) with normal alveolar spaces and thin interalveolar septa (S). (H&E X 200)

**Fig (10):** A photomicrograph of a lung section from subgroup IIIc (virgin olive oil-treated) showing alveoli lined with type I pneumocytes (thin arrows) and type II pneumocytes (thick arrows) with thin interalveolar septa (S). Notice alveolar macrophages (arrow heads), normal blood vessels (V), focal area of mild interstitial mononuclear cellular infiltration (F) and hemorrhage (h). (H&E X 400)
with dark flat pyknotic nuclei. However, type II pneumocytes were frequently seen with partially loss of their surface microvilli. They revealed vacuolated cytoplasm with lamellated bodies having variable electron densities. Some of them were empty, while others have irregular electron dense content (Fig. 13). Numerous alveolar macrophages with large, indented nuclei and many lysosomes were frequently encountered within the alveolar lumen. Interstitial mononuclear cellular infiltration as well as deposition of collagen fibrils were also observed (Figs. 13,14).

Subgroup IIIa (animals treated with gentamicin): Ultrathin sections of lung specimens revealed non observable improvement. Type I pneumocytes were scanty and showed irregular dark nuclei and indistinct vacuolated cytoplasm. Type II pneumocytes still frequently seen and their cytoplasm contained many lamellated bodies with variable electron densities, however some vacuolated and empty bodies were also observed (Figs. 15,16). Multinucleated macrophages with many lysosomes as well as interstitial mononuclear cellular infiltration observed (Fig. 16).

Subgroup IIIb (animals treated with gentamicin and virgin olive oil): Ultrathin sections of lung specimens showed that addition of olive oil had a favorable effect with a considerable degree of preservation of alveolar epithelial lining. Type II pneumocytes were less frequent as compared with groups II and IIIa and their cytoplasm contained numerous lamellated bodies with concentric or parallel lamellae (Fig. 17). Moreover, flattened type I pneumocytes were frequently appeared with flat large nuclei. However, interstitial mononuclear cellular infiltration with deposition of collagen fibrils and alveolar macrophages were still observed (Figs. 17,18). Macrophages appeared as large cells with many pseudopodia on their surfaces and contained large ovoid nuclei with prominent nucleoli and many cytoplasmic lysosomes (Fig. 18).

Subgroup IIIc (animals treated with virgin olive oil only): Ultrathin sections of lung specimens revealed that lung architecture become apparently normal and resemble the control group. The alveoli appeared lined predominantly with the flattened type I pneumocytes with flat nuclei and the interspersed large cuboidal type II pneumocytes with rounded euchromatic nuclei and short microvilli on their cell surface. Their cytoplasm contained mitochondria and numerous lamellated bodies with concentric or parallel lamellae (Figs. 19,20). However, mild interstitial mononuclear cellular infiltration with deposition of collagen fibrils were still observed (Fig. 20).

Immunohistochemical results:

**Group I (subgroups Ia and Ib):** Lung sections

**Explanation of figures**

**Fig (11):** An electron micrograph of a lung section from group I (control) showing type I pneumocyte (PI) with a flat nucleus (NI) filling a large part of the cytoplasm and type II pneumocyte (PII) is cuboidal in shape with few short microvilli on their surface (thick arrow). Notice large rounded nucleus (NII), mitochondria (M) and numerous cytoplasmic lamellated bodies (L) with concentric or parallel lamellae. (TEM X 6000)

**Fig (12):** An electron micrograph of a lung section from group I (control) showing type I pneumocyte (PI) with flat nucleus (NI) and smooth surface (thin arrow). Notice an alveolar macrophage (P) with a large ovoid intended nucleus (arrow head) and the cytoplasm contains few organelles with some lysosomes (Ly). Notice pseudopodia on the cell surface (thick arrow). (TEM X 5000)

**Fig (13):** An electron micrograph of a lung section from group II (induced-lung sepsis) showing type I pneumocyte (PI) with pyknotic nucleus (NI) and type II pneumocytes (PII) with partial loss of microvilli (arrow heads ) and vacuolated empty lamellar bodies (L). Notice alveolar macrophages (P) with many lysosomes (Ly) in their cytoplasm and interstitial deposition of some collagen fibrils (arrows). (TEM X 6000)

**Fig (14):** An electron micrograph of a lung section from group II (induced-lung sepsis) showing type I pneumocytes (PI) with dark pyknotic nucleus (NI) and two alveolar macrophages (P) with many lysosomes (Ly) and irregular nuclear outline (arrow heads). Notice the interstitial deposition of some collagen fibrils (arrow) and interstitial mononuclear cellular infiltration (F). (TEM X 6000)

**Fig (15):** An electron micrograph of a lung section from subgroup IIIa (gentamicin-treated) showing type I pneumocyte (PI) with elongated dark nucleus (NI). Many type II pneumocytes (PII) with irregular oval nuclei (NII) and lamellated bodies of variable electron densities (L). Some of them are vacuolated (V). Notice interstitial mononuclear cellular infiltration (F). (TEM X 5000)

**Fig (16):** An electron micrograph of a lung section from subgroup IIIa (gentamicin-treated) showing type I Pneumocyte (PI) with irregular nucleus (NI), destroyed mitochondria (M) and cytoplasmic vacuoles (V). Type II pneumocytes (PII) contain lamellar bodies with variable electron densities (L) are seen. Notice interstitial cellular infiltration (F) and multinucleated macrophage (P) with many lysosomes (Ly). (TEM X 5000)
Fig (17): An electron micrograph of a lung section from subgroup IIIb (gentamicin and virgin olive oil-treated) showing two type II pneumocytes (PII), one of them with irregular dark intended nucleus (NII) and numerous lamellar bodies of variable electron densities and parallel lamellae (L). Notice interstitial mononuclear cellular infiltration (F) with deposition of interstitial collagen fibrils (arrow). (TEM X 4000)

Fig (18): An electron micrograph of a lung section from subgroup IIIb (gentamicin and virgin olive oil-treated) showing type I pneumocyte (PI) with irregular dark nucleus (NI), alveolar macrophage (P) with a large ovoid nucleus (N) and prominent nucleolus and multiple pseudopodia on the cell surfaces (arrow heads). The cytoplasm contains few organelles and many lysosomes (Ly). Notice type II pneumocyte (PII) with surface microvilli (arrow) and lamellar bodies (L). (TEM X 6000)

Fig (19): An electron micrograph of a lung section from subgroup IIIc (virgin olive oil-treated) showing two type I pneumocytes (PI) with large oval nuclei (NI) and prominent nucleoli and one type II pneumocyte (PII) with round intended nucleus (NII), mitochondria (M), lamellar bodies of variable electron densities (L) and short microvilli on the cell surface (thin arrow). Notice few inflammatory cells (F). (TEM X 5000)

Fig (20): An electron micrograph of a lung section from subgroup IIIc (virgin olive oil-treated) showing type I pneumocyte with a flat oval nucleus (NI) filling a large part of the cytoplasm. Type II pneumocyte (PII) with a large rounded nucleus (NII), lamellar bodies of variable electron densities (L) and short microvilli on the cell surface (thick arrow). Notice few inflammatory cells infiltration (F) with deposition of interstitial collagen fibrils (thin arrow). (TEM X 5000)
revealed negative immune reaction to HSP70 almost in all fields (Fig. 21). Group II (Induced-lung sepsis): Lung sections showed intense positive immune reaction in the form of brownish cytoplasmic granules, these granules were observed in type I and type II pneumocytes as well as mononuclear interstitial cells (Fig. 22). Subgroup IIIa (animals treated with gentamicin): Lung sections showed intense positive immune reaction in the lining epithelium of the alveoli as well as the inflammatory cells (Fig. 23). Subgroup IIIb (animals treated with gentamicin and virgin olive oil): lung sections showed positive immune reaction in few pneumocytes and mononuclear interstitial cells (Fig. 24). Subgroup IIIc (animals treated with virgin olive oil only): showed almost negative immune reaction in the lining epithelial cells of alveoli as compared with control group. While, few positive cells in the interstitial tissue were observed (Fig. 25).

**Morphometric analysis (Table 1, Histogram I):** The mean color intensity of HSP70-positive immunoreaction in the group II was (16.48 ± 5.27*) and in the subgroup IIIa was (15.47±3.46*) showed a significant increase (P<0.001) as compared with control group (7.45 ± 2.13). Whereas the subgroup IIIb (10.96±3.12*) showed a significant increase (P<0.05) as compared with control group. While the mean color intensity of HPS70 in subgroup IIIc showed a non-significant change (7.82±1.84) as compared with control group.

**Statistical analysis of body weight and hemodynamic parameters results:** All experimental groups revealed that there was no statistical significance difference with p-value > 0.05 between different study groups at the base line of study (before treatment) as regards to body weight, systolic (SBP), diastolic (DBP), mean arterial blood pressure (MAP) and also for heart rate (HR), which indicates proper matching between study groups.

**Control group I (both subgroups: Ia and Ib):** Revealed no statistical significance difference with p-value >0.05 between both of subgroups at the end line of study as regards to weight, blood pressure and heart rate.

**Statistical analysis of body weight results :** On the mean of body weight before and after the experiments in each group, it was found that lung-sepsis rats (group II) and the gentamicin-treated (subgroup IIIa) showed a significant decrease (P<0.001) in body weight when compared to their weight before treatment as well as gentamicin and virgin olive oil-treated group (subgroup IIIb). Whereas virgin olive oil-treated group (subgroup IIIc) revealed a non significant decrease (P>0.05) when compared to their body weight before treatment (Table 2).

On the other hand, a comparison of the percentage of change of body weight for each group with control group revealed significant decrease (P<0.001). However, No significant difference (P>0.05) could be seen between percentage of change in all treated groups (subgroup IIIa, IIIb and IIIc) when compared to lung sepsis group (group II) (Table 2).

**Statistical analysis of hemodynamic results:** In lung sepsis (group II), there was a significant decrease (P< 0.001) in mean arterial blood pressure associated with a significant increase (P< 0.001) in heart rate when compared to control group. On comparison with control group, all treated groups (subgroups IIIa, IIIb and IIIc) showed a significant decrease (P<0.001) in mean arterial blood pressure with non significant difference (P>0.05) in heart rate (Table 3).

Treatment with gentamicin (subgroup IIIa) showed a non significant change in both mean arterial blood pressure and heart rate (P>0.05) when compared to group II. However, concomitant treatment with virgin olive oil and gentamicin (subgroup IIIb) as well as treatment with virgin olive oil only (subgroup IIIc) showed improvement of both blood pressure and heart rate. Whereas, the mean arterial blood pressure were significantly increased (P<0.05) associated with a significant decreased (P<0.05) in heart rate as compared to group II (Table 3).

**DISCUSSION**

Lung is a major target organ for sepsis, which is a most important worldwide public health problem. A safe and effective mode of treatment is required to manage lung diseases before development of septic shock with a significant drop in blood pressure that can lead to death. Antibiotics alone are not an efficient treatment plan to increase the possible survival rate of the septic patient. It is suggested that the use of antioxidants may have an important role
Figs. 21 to 25 represent photomicrographs of lung sections demonstrating immunoreaction.
in the treatment of septic patients [5,18].

This study demonstrates that, all rats of the induced-lung sepsis group showed significant blood pressure reduction with an increase of heart rate. Hypotension can be explained by the induction of NOS-II with consecutive formation of high amounts of NO in nearly all organs. This vasodilatory molecule is responsible for the cardiovascular failure in all septic and endotoxic shock states as indicated by the elevated plasma and tissue levels of nitrate/nitrite concentration [25,26].

In this work, the light microscopic studies of lung sepsis (group II) revealed, prominent alterations of normal structure and architecture of the lung tissue manifested by the appearance of over expanded alveoli with thinning of their walls, destruction of the inter-alveolar septa and connection of many alveoli together, side by side with areas of collapsed alveoli and thickened inter-alveolar septa. Attenuation and exfoliation of the lining epithelium of the bronchiolar walls were also observed. This coincided with other workers [27,28], who discovered that inflammatory lung injury induced by lipopolysaccharide (LPS) demonstrates vascular damage with increased epithelial barrier permeability and breakdown of connective tissue giving the picture of emphysema with sever hypoxemia. The hallmark of lung sepsis is excessive influx of inflammatory cells into the lung tissue and high-protein interstitial edema with diffuse alveolar damage [29]. They also reported that apoptosis of the superficial respiratory epithelium and lining epithelium of the intrapulmonary air passages is a consequence of blood vessels damage during lung sepsis [28].

Ultrastructurally, this study revealed reduction in type I pneumocytes with proliferation and atypical vacuolation of type II pneumocytes with degenerative changes in their lamellar bodies leaving irregular empty spaces. These findings were in agreement with previous reports of some investigators [30]. Who reported that sepsis cause acute lung injury with increased activation of cytokines and inflammatory mediators which are produced in the endothelial cells within the pulmonary vasculature. These cytokines induced loss of the integrity of pulmonary vascular endothelial cells and loss of pulmonary epithelial cells, particularly type I pneumocytes [31]. Other experimental studies suggested that type II pneumocytes might constitute the reserve epithelial cells of the alveoli, and its proliferation and hyperplasia reflected underlying injury of type I pneumocytes and were regarded as a manifestation of their repair [32].

In this study, appearance of many active alveolar macrophages with presence of inflammatory cells may be attributed to their important role in the lung defense [33]. Altered surfactant metabolism in early sepsis-induced lung injury was found to be the main factor contributing to type II pneumocytes changes with decreased conversion of large aggregates into small aggregates and an increased uptake of surfactant into alveolar macrophages [34]. In the present study, deposition of collagen fibers in the inter-alveolar and perivascular tissues was confirmed by electron microscopic examination. This finding was in agreement with other researchers who stated that lung extracellular matrix composition is dramatically altered in response to acute lung injury with the destruction of alveolar basement membrane.

Explanation of figures

**Fig (21):** Photomicrograph of a lung section from group I (control) showing negative immune reaction to HSP70 in the lining epithelium of the alveoli. (HSP70 X1000)

**Fig (22):** Photomicrograph of a lung section from group II showing intense positive immune reaction to HSP70 in the lining epithelium of the alveoli, intense brown granules in the cytoplasm of type I (thin arrow) and type II (thick arrow) pneumocytes, as well as in the cytoplasm of mononuclear cellular infiltration (arrow heads). (HSP70 X1000)

**Fig (23):** Photomicrograph of a lung section from subgroup IIa showing focal areas of lining epithelium of the alveoli with a positive immune reaction to HSP70, intense brown granules in the cytoplasm of some of type I (thin arrows) and type II (thick arrow) pneumocytes as well as in the cytoplasm of mononuclear cellular infiltration (arrow heads). (HSP70 X1000)

**Fig (24):** Photomicrograph of a section from lung rat of subgroup IIIb showing few cells in the lining epithelium of the alveoli (arrows) with a positive immune reaction to HSP70. Notice positive immune reaction in few cells in the interalveolar septa (arrow heads). (HSP70 X1000)

**Fig (25):** Photomicrograph of a section from lung rat of subgroup IIIc showing negative immune reaction to HSP70 in the lining epithelium of the alveoli. One cell in the interalveolar septa (arrow head) showed positive immune reaction to HSP70. (HSP70 X1000)
followed by increased deposition of fibronectin and type I collagen by alveolar epithelial cells and fibroblasts [35].

In gentamicin-treated group no significant changes observed in body weight, blood pressure and heart rate as compared with lung sepsis group. The most observable histological changes were the preserved alteration of lung tissue that was previously observed in lung sepsis. This could be explained by Martinez-Salgado et al. and Suganya et al. [11,36] who reported that gentamicin enhance the generation of reactive oxygen species (ROS) with misbalances of the antioxidant status. Abnormal production of ROS may result in cellular injury and necrosis through peroxidation of membrane lipids, protein denaturation and DNA damage [37]. Furthermore, gentamicin induced toxicity by different pathological mechanisms as apoptosis, necrosis, elevation of endothelin I, and increase of monocytes and macrophages infiltration. Activation of alveolar macrophages could release many mediators such as tumor necrotizing factor, which augment the inflammatory response of airways and alveoli [38,39].

Concomitant administration of virgin olive oil with gentamicin in the present study, showed a considerable improvement of damage of lung tissue and architecture. However, few alveoli were still collapsed and inter-alveolar septa were mildly thickened with mild cellular infiltration. This result is in consonance with De la Puerta et al. [40], who reported that olive oil is one of the most potent natural antioxidants and reversed tissue damage by preventing the decline of antioxidant defense system. In addition it has a capacity of elimination of free radical superior to the antioxidant capacity of the vitamin C and E [41].

On the other hand, virgin olive oil-treated (subgroup IIIc) showed improving body weight, hemodynamic parameters with a considerable improvement of the lung architecture. This was in agreement with Preedy and Watson [42], who reported that immunonutrition with olive oil reduced the release of the inflammatory mediators, pro-inflammatory cytokine as well as the induced alteration of septic damage of lung alveoli. It has been reported that olive oil-derived phenolic compounds including oleuropein have the ability to scavenge nitric oxide (NO) and promote the expression of the inducible nitric oxide synthase (iNOS) in the cells [40].

In this experimental study, the HSP70 expression revealed significant increase in rats with lung sepsis and gentamicin-treated alone as well as gentamicin with virgin olive oil–treated animals. While, there was non-significant changes of HSP70 expression in rats treated with virgin olive oil only as compared with control group. These finding was coincided with the other researches [43], who explained that increase HSP70 in serum during sepsis is important to protect the body from excessive damage. The HSP70 is closely associated with pulmonary biology and has protective effects on lung injury due to their anti-inflammatory, anti-oxidation and molecular chaperone roles [44]. Moreover, HSP70 has the ability to alter apoptosis, which limits over-proliferation of type II pneumocytes and preserves type I alveolar epithelial cells [8,44].

In conclusion, the most remarkable results in this study were obtained in treatment with virgin olive oil alone. So, it is recommended that awareness should be focused on virgin olive oil as an important protective and regenerative against oxidative stress in the lung damage.

ACKNOWLEDGEMENTS

Authors are grateful to College of Medicine, King Faisal University, Saudi Arabia for providing all facilities and equipment to complete this work.

REFERENCES
