Effects of Statins Versus Corticosteroid and their Combination on the Modulation of Acute Lung Injury in Rats: Comparative Study

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Abstract

Background: Acute Lung Injury (ALI) is an extremely important health problem. It affects all age groups and has a high mortality up to 30-50%. The pleiotropic properties of statins, including anti-inflammatory, immuno-modulatory and anti-oxidant effects could be promising in the treatment of ALI.

Aim of the Work: The aim of the present work was to compare the effect of statins versus corticosteroid and their combination in the modulation of ALI.

Methods: 162 male albino rats were subdivided into normal group, Lipopolysaccharide (LPS) treated rats (model of ALI), saline treated groups, ALI statins (simvastatin, rosuvastatin) treated groups, ALI methylprednisolone treated group, and ALI combination therapy of statins and methylprednisolone. Lung tissue myeloperoxidase, malondialdehyde, and total lung proteins were assessed at 24 hours, 72 hours and on the 7th day after induction of ALI. Histopathological examination of the left lungs of all animals in all groups was done all through the experimental periods.

Results: The lipopolysaccharide induced ALI group showed significant increase in myeloperoxidase, malondialdehyde, total lung proteins and pathological score. Treatment of rats with ALI with statins or methylprednisolone resulted in significant decrease in these elevated parameters at 24 hours, 72 hours and on the 7th day as compared to the untreated model group. Methylprednisolone therapy alone was significantly effective than simvastatin therapy alone in reducing myeloperoxidase, malondialdehyde, total lung proteins and pathological score. Rosuvastatin was more significantly effective in reducing the level of myeloperoxidase, malondialdehyde, total lung proteins and histopathological score measured at the end of the present study than simvastatin therapy. Regarding the combination therapy, methylprednisolone with rosuvastatin showed the most significant reduction of all parameters all through the experiment.

Conclusion: A significant improvement and amelioration of ALI biochemical and pathological changes could be achieved by treatment either with statins alone or in combination with methylprednisolone. The improvement was more apparent with rosuvatatin and methylprednisolone combination therapy.

Key Words: ALI – Statins – Methylprednisolone – Combination therapy – Myeloperoxidase – Malondialdehyde – Total lung proteins – Histopathological score.

Introduction

ACUTE Lung Injury (ALI) is a clinical syndrome characterized by tachypnoea, hypoxaemia resistant to supplemental oxygen, diffuse alveolar infiltrates, and decreased pulmonary compliance. It occurs in response to a variety of local and systemic insults, such as trauma and severe sepsis. It affects all age groups and has a high mortality up to 30-50%. The high incidence, mortality, long-term consequences and high economic costs mean that ALI is an extremely important health problem [1].

Infiltration of the lung with neutrophils and proinflammatory cytokines in serum and Bronchoalveolar Lavage Fluid (BALF), and released toxic mediators such as reactive oxygen species are important features of the inflammatory response that characterizes ALI [2].

Several lines of evidence suggest a critical role for the neutrophil in the pathogenesis of most cases of ALI. Histological studies of early ALI consistently show a marked accumulation of neutrophils in the lung. Pulmonary edema fluid and bronchoalveolar lavage fluid from ALI/ARDS patients also have a predominance of neutrophils [3].

Increased generation of Reactive Oxygen Species (ROS), derived largely from activated Polymorphnuclear (PMN), may result in DNA, protein, and lipid oxidation and thus contribute to the
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degradation of the lung parenchyma [4]. Although the lung is provided with a variety of antioxidant enzymes and substrates [e.g., Glutathione (GSH) peroxidase, Superoxide Dismutase (SOD), catalase, alkenyl-acyl phosphatidylcholine in pulmonary surfactant, Vitamins E, A, and C] and may even be enriched with these protective species (e.g., 100-fold higher GSH concentration in the alveolar lining layer as compared with blood), these radical scavenger systems may not sufficient to neutralize the immense burden of ROS produced under conditions of ARDS [5].

The uncontrolled local inflammatory response in ALI contributes to alveolar epithelial and capillary endothelial barrier damage, increased alveolar capillary membrane permeability, and exudation of protein-rich edema fluid into the alveolar space leading to the development of pulmonary edema [6].

The goal of treatment of ALI is to prevent further lung injury, reduce lung edema, and maintain tissue oxygenation. Treatment may be non-pharmacologic or pharmacologic. Various therapies have been evaluated, but none has definitively demonstrated efficacy. Clinical trials targeting pharmacotherapies have failed to show a reduction in mortality. Currently, the only treatment with proven efficacy is the nonpharmacologic use of lung-protective mechanical ventilation [7]. However, multiple novel therapies have emerged as promising candidates for trials of prevention or early treatment of ALI.

Statins are among the most widely prescribed classes of medicines in the world. They have ranked among the best studied medications [8]. The pleiotropic properties of these drugs, including anti-inflammatory, immuno-modulatory and antioxidant effects could be promising in the treatment of ALI [9].

The aim of the present work was to compare the effect of statins versus corticosteroid and their combination in the modulation of ALI.

Material and Methods

Animals:

The study included 162 laboratory bred male adult albino rats kept in the Animal House of Veterinary College in Beni-Suef University, weighing 150-250gm. They were maintained at 25°C, normal light/dark cycle (12 hours dark/12 hours light). They were fed standard chow diet. This work started on 2011 and ended on 2015. All animals were handled according to the guidelines of the local ethical committee which comply with the international laws for use and care of laboratory animals.

Drugs:
- Methylprednisolone powder (Pfizer, USA).
- Rosuvastatin (Crestor) tablet 20mg (Astra-zenca, England).
- Simvastatin (Alkor) tablet 40mg (Alcan, Canada).

Chemicals:
- Lipopolysaccharide of Escherichia Coli (055: B5) (Sigma-Aldrich).

Reagents for measurement of biochemical parameters:
- Malondialdehyde ELISA kit: (OxisResearchTM A Division of OXIS Health Products, Inc.).
- Myeloperoxidase ELISA kit: (OxisResearchTM A Division of OXIS Health Products, Inc.).
- Total proteins kit: (Spectrum diagnostics, Egypt).

Experimental design:

The study was divided into 3 time periods (24 hours, 72 hours and 7 days) after the administration of LPS to induce acute lung injury. At each time point, there were 9 groups (6 rats each).

Group 1: Included normal rats and served as control.

Group 2: Rats received lipo-polysaccharide (LPS) of E.Coli in a dose of 100 gg/rat dissolved in 100g/l saline instilled intra-nasally once [10]. This group served as a model of acute lung injury.

Group 3: Rats received 1ml saline, given orally for 7 days starting 3 hours after LPS administration and served as control for the groups that receive the tested drugs.

Group 4: Rats received 0.1ml saline intra-venously in the rat tails for 7 days starting 3 hours after LPS administration. This group served as control for the methylprednisolone treated group.

Group 5: Rats received simvastatin dissolved in saline (15mg/kg/day) orally [11] for seven days starting three hours after LPS administration.

Group 6: Rats received rosuvastatin dissolved in saline (15mg/kg/day) orally for seven days starting three hours after they were subjected to LPS.

Group 7: Rats received methylprednisolone dissolved in saline (2mg/kg/day) intra-venously in the rat tails for seven days starting three hours after LPS administration.
**Group 8:** Rats received a combination therapy of simvastatin and methylprednisolone in the previous doses, duration and routes of administration starting three hours after LPS administration.

**Group 9:** Rats received a combination therapy of rosuvastatin and methylprednisolone in the previous doses, duration and routes of administration starting three hours after LPS administration.

**Induction of ALI:**

The rats were anesthetized and each rat received Escherichia coli O55:B5 LPS (Sigma Aldrich) in a dose of 100 µg/rat dissolved in 100 µl saline by intranasal instillation by use of the external nares route until the solution was inhaled. The animals were allowed to recover from anesthesia, returned to their cages, and were given free access to water and food. After three hours, treatment with simvastatin, rosuvastatin, methylprednisolone or combination therapy was given to the animals daily and continued for seven days.

The right lungs of the animals in all the groups were removed and subjected to homogenization. Measurement of the following biochemical parameters in the homogenates were done at 24 hours, 72 hours, and at the end of the experiment.

- Myeloperoxidase (MPO) the indicator of neutrophil extravasation.
- Malondialdehyde, the indicator of lipid peroxidation.
- Total protein concentration.

The left lungs of the animals in all the groups were removed and subjected to histopathological examination at 24 hours, 72 hours and at the end of the experimental period. For routine histopathological examination each case was stained with Hematoxylin-Eosin stain and examined under the light microscope.

Pathological score was done with the following criteria: Neutrophilic infiltration, hemorrhage, congestion, thickening of alveolar wall, airway epithelial damages and interstitial edema was done. It was graded from 0-4 as follows: 0 for normal, 1 for mild, 2 for moderate, 3 for severe and 4 for massive changes for each one of the criteria [12].

**Statistical methods:**

The data was collected, coded and entered to computer. The data was analyzed with the program (SPSS) Statistical Package for Social Science Version 16 under Windows 7.

**Results**

**A- Effects of the tested drugs on the mean (±SD) level of myeloperoxidase (U/gm) in lung tissue homogenate:**

The mean (±SD) level of myeloperoxidase in lung tissue homogenate obtained from normal rats were 3.52±0.50761, 3.50±0.55 and 3.50±0.55 after 24 hours, 72 hours and at the end of the experiment respectively (Table 1).

Induction of ALI in rats (group 2) significantly elevated the myeloperoxidase levels in their lung tissue homogenate compared to normal rats (group 1) (Table 1) all through the experimental periods.

Oral or IV administration of saline (control groups 3,4) for rats with ALI did not produce significant changes of the elevated levels of myeloperoxidase in the untreated model group (2) all through the experiment (Table 1).

Treatment of rats with ALI with either simvastatin, rosuvastatin or methylprednisolone caused a significant decrease in the mean levels of lung tissue myeloperoxidase all through the experimental periods when compared to the untreated model group. The mean levels of lung tissue myeloperoxidase in the simvastatin treated groups, rosuvastatin treated group and in the methylprednisolone treated group at all the experimental periods were shown in Table (1).

The percentage reduction of the mean myeloperoxidase level with simvastatin was 20.8%, 35.3% and 46.1% and with rosuvastatin 32.2%, 54.8% and 61.5% after 24 hours, 72 hours, and 7 days respectively. It was observed that the percentage reduction was more apparent with time. The percentage reduction with methylprednisolone was 32.2%, 53.9% and 65.3% after 24 hours, 72 hours, and 7 days respectively.

The mean level of myeloperoxidase was significantly reduced more with rosuvastatin than with simvastatin after 24 hours, after 72 hours and at the end of the experiment (Table 1). It was also noticed that the reduction in the level of this pa-
rameter was significantly reduced more with methylprednisolone than with simvastatin all through the experiment (Table 1).

The effect of combination therapy of simvastatin and methylprednisolone as well as combination therapy of rosuvastatin and methylprednisolone to rats with ALI also caused significant reduction in the mean levels of lung tissue myeloperoxidase at all time periods when compared to the untreated model group (Table 1). The percentage reduction was 45.8%, 55.75% and 66.9% with combination therapy of simvastatin and methylprednisolone and 61.4%, 66.3% and 73.8% in combination therapy of rosuvastatin and methylprednisolone after 24 hours, 72 hours, and 7 days respectively.

It was noticed that simvastatin and methylprednisolone combination therapy produced more significant decrease of the elevated level of myeloperoxidase when compared to treatment with simvastatin alone all through the experiment, and when compared to either methylprednisolone alone or rosuvastatin alone at 24 hours (Table 1).

Rosuvastatin and methylprednisolone combination therapy produced significant reduction of increased level of myeloperoxidase when compared to therapy either with simvastatin or rosuvastatin alone at all time periods. Moreover, significant reduction was observed when compared to methylprednisolone either alone or in combination with simvastatin at all time periods (Table 1).

Table (1): Effects of the tested drugs on the mean (±SD) levels of myeloperoxidase (U/gm) in lung tissue obtained from rats with ALI.

<table>
<thead>
<tr>
<th>Groups</th>
<th>–24 hours after induction of ALI</th>
<th>–72 hours after induction of ALI</th>
<th>–7 days after induction of ALI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rats (group 1).</td>
<td>3.52±0.51</td>
<td>3.50±0.55</td>
<td>3.50±0.55</td>
</tr>
<tr>
<td>Model of ALI (group 2).</td>
<td>9.67±0.52a</td>
<td>11.33±0.82a</td>
<td>13±1.26a</td>
</tr>
<tr>
<td>ALI + saline oral (1ml/day) (group 3).</td>
<td>9.17±1.17a</td>
<td>11.50±1.05a</td>
<td>12.50±2.26a</td>
</tr>
<tr>
<td>ALI + saline IV (0.1ml/day) (group 4).</td>
<td>9.83±1.60a</td>
<td>12.00±1.10a</td>
<td>13.33±1.75a</td>
</tr>
<tr>
<td>ALI + simvastatin (15mg/kg/day) (group 5).</td>
<td>7.67±0.82b</td>
<td>7.33±0.82b</td>
<td>7±0.89b</td>
</tr>
<tr>
<td>ALI + rosuvastatin (15mg/kg/day) (group 6).</td>
<td>6.50±0.55bc</td>
<td>5.16±0.75bc</td>
<td>5.00±0.89bc</td>
</tr>
<tr>
<td>ALI + methylprednisolone (2mg/kg/day) (group 7).</td>
<td>6.50±0.55bc</td>
<td>5.25±0.30bc</td>
<td>4.52±0.30bc</td>
</tr>
<tr>
<td>ALI + combined simvastatin + methylprednisolone (group 8).</td>
<td>5.20±0.24bcde</td>
<td>5.00±0.63bc</td>
<td>4.33±0.82bc</td>
</tr>
<tr>
<td>ALI + combined rosuvastatin + methylprednisolone (group 9).</td>
<td>3.71±0.17bcdef</td>
<td>3.85±0.14bcdef</td>
<td>3.40±0.26bcdef</td>
</tr>
</tbody>
</table>

The data expressed as (mean ± S.D) (n=6).
a: Significant as compared to normal (p<0.05).
b: Significant as compared to untreated model (p<0.05).
c: Significant as compared to simvastatin alone (p<0.05).
d: Significant as compared to rosuvastatin alone (p<0.05).
e: Significant as compared to methylprednisolone alone (p<0.05).
f: Significant as compared to combined simvastatin and methylprednisolone (p<0.05).

B- Effects of the tested drugs on the mean (±SD) level of malondialdehyde (nmol/gm) (as an oxidative stress marker) in lung tissue homogenate:

The mean (±SD) level of malondialdehyde as a marker of oxidative stress in lung tissue homogenate obtained from normal rats were 31.16 ± 1.17 (ng/gm) after 24 hours, 33.00±3.58 and 32.00±4.90, at 72 hours and at the end of the experiment, respectively.

Induction of ALI in rats (group 2) significantly elevated the malondialdehyde levels in their lung tissue homogenate compared to normal rats (group 1) at all the experimental periods (Table 2).

Oral or IV administration of saline (control groups 3, 4) for rats with ALI did not produce significant change of the elevated levels of malondialdehyde in the untreated model (group 2) all through the experiment (Table 2).

Treatment of rats with ALI with either simvastatin, rosuvastatin, or methylprednisolone decreased significantly the high levels of malondialdehyde all through the experiment, when compared to the untreated model group (Table 2).

The percentage reduction with simvastatin was 43.5%, 52% and 58.1% and with rosuvastatin...
51.9%, 58.6% and 64.3% after 24 hours, 72 hours, and 7 days. It was noticed that the percentage reduction of this elevated levels was more apparent with time. Percentage reduction with methylprednisolone was 51.5%, 60.66% and 63.75%, after 24 hours, 72 hours, and 7 days respectively.

The mean level of malondialdehyde was significantly reduced more with rosuvastatin therapy than with simvastatin at 24 hours, 72 hours and at the end of the experiment (Table 2).

It was noticed that the reduction in the level of this parameter was significant with methylprednisolone than simvastatin at 24 hours, 72 hours, and at the end of the experiment (Table 2).

Regarding the combination therapy, the combination therapy of simvastatin and methylprednisolone as well as the combination therapy of rosuvastatin and methylprednisolone in rats with ALI also caused significant reduction in the mean levels of lung tissue malondialdehyde at all time periods when compared to the untreated model (Table 2).

The percentage reduction was 58.7%, 64% and 69.3% in combined therapy of simvastatin and methylprednisolone, and 61%, 69.3%, 78.1% in combined therapy of rosuvastatin and methylprednisolone after 24 hours, 72 hours, and 7 days respectively.

It was noticed that simvastatin and methylprednisolone combination therapy produced significant decrease of the elevated level of malondialdehyde when compared to treatment with either simvastatin alone or rosuvastatin alone all through the experiment and to methylprednisolone alone at 24 hours and at end of the experiment (Table 2).

Rosuvastatin and methylprednisolone combination therapy produced significant reduction of increased level of malondialdehyde when compared to therapy either with simvastatin or rosuvastatin alone. Moreover, significant reduction was observed when compared to methylprednisolone alone either alone all through the experiment or in combination with simvastatin at 72 hours and at the end of the experiment (Table 2).

### Table 2: The effects of the tested drugs of the study on the mean (±SD) levels of malondialdehyde (nmol/gm) in lung tissue obtained from rats with ALI.

<table>
<thead>
<tr>
<th>Groups</th>
<th>–24 hours</th>
<th>–72 hours</th>
<th>–7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rats (group 1).</td>
<td>31.16±1.17</td>
<td>33.00±3.58</td>
<td>32.00±4.90</td>
</tr>
<tr>
<td>LPS (model) (group 2).</td>
<td>13.17±2.40</td>
<td>15.17±4.80</td>
<td>16.07±11.25</td>
</tr>
<tr>
<td>ALI + saline oral (1ml/day) (group 3).</td>
<td>129.00±2.1a</td>
<td>148.17±5.31a</td>
<td>154.67±8.66a</td>
</tr>
<tr>
<td>ALI + saline IV (0.1ml/day) (group 4).</td>
<td>133.17±3.66a</td>
<td>152.17±4.02a</td>
<td>163.67±10.54a</td>
</tr>
<tr>
<td>ALI + simvastatin (15mg/kg/day) (group 5).</td>
<td>74.16±3.06b</td>
<td>72.00±2.1b</td>
<td>67.16±1.7b</td>
</tr>
<tr>
<td>ALI + rosuvastatin (15mg/kg/day) (group 6).</td>
<td>63.16±1.72bc</td>
<td>62.66±0.82bc</td>
<td>57.16±1.94bc</td>
</tr>
<tr>
<td>ALI + methylprednisolone (2mg/kg/day) (group 7).</td>
<td>64.50±5.82bc</td>
<td>59.66±3.56bc</td>
<td>58.33±2.16bc</td>
</tr>
<tr>
<td>ALI + combined simvastatin + methylprednisolone (group 8).</td>
<td>54.16±3.76bcd</td>
<td>54.00±6.42bcd</td>
<td>49.66±2.80bcd</td>
</tr>
<tr>
<td>ALI + combined rosuvastatin + methylprednisolone (group 9).</td>
<td>51.83±4.62bcdef</td>
<td>46.16±2.56bcdef</td>
<td>35.66±1.75bcdef</td>
</tr>
</tbody>
</table>

The data expressed as (mean ± S.D) (n=6):
- a: Significance as compared to normal (p<0.05).
- b: Significance as compared to untreated model (p<0.05).
- c: Significance as compared to simvastatin alone (p<0.05).
- d: Significance as compared to rosuvastatin alone (p<0.05).
- e: Significance as compared to methylprednisolone alone (p<0.05).
- f: Significance as compared to combined simvastatin and methylprednisolone (p<0.05).

### C- Effects of the tested drugs on the mean (±SD) level of proteins (g/g) in lung tissue homogenate (as a marker of pulmonary oedema):

The mean levels of proteins in lung tissue homogenate obtained from normal rats were mean (±SD) 0.50±0.07 (g/g), 0.60±0.07 and 0.50±0.05 at 24, 72 hours and at the end of the experiment, respectively (Table 3).

Induction of ALI in rats (group 2) significantly elevated the proteins levels in their lung tissue homogenate compared to normal rats group (1) all through the experimental periods (Table 3).

Oral or IV administration of saline (control group 3, 4) for rats with ALI did not produce any significant change of the elevated levels of lung proteins in the untreated model (group 2) all through the experiment (Table 3).

Treatment of rats with ALI with either simvastatin, rosuvastatin, or methylprednisolone caused
a significant decrease in the mean level of lung tissue protein when compared to the untreated model group at 72 hours and at the end of the study. The mean levels of tissue protein in simvastatin treated group were 1.2±0.18, 1.00±0.16 and 0.95±0.03, and in the rosuvastatin group were 1.18±0.05, 0.83±0.04, and 0.76±0.07g/g and in the methylprednisolone 1.30±0.17 at 24 hours, 1.14±0.16 at 72 hours and 0.8200±0.07 at the end of the experiment (Table 3).

The percentage reduction of the mean lung tissue proteins with simvastatin treatment was 41.1% at 72 hours and 53.6% at the end of the experiment when compared to methylprednisolone combination therapy produced significant reduction of the elevated levels of lung tissue proteins when compared to treatment with either simvastatin and methylprednisolone respectively. It was noticed that simvastatin and methylprednisolone combination therapy produced significant decrease of the elevated levels of lung tissue proteins when compared to therapy with either simvastatin alone at 72 hours and at the end of the experiment or methylprednisolone alone at 72 hours.

Rosuvastatin and methylprednisolone combination therapy produced significant reduction of the increased lung tissue levels of proteins when compared to therapy with either simvastatin alone all through the experiment or rosuvastatin alone at 24, 72 hours. Moreover, significant reduction was observed when compared to methylprednisolone either alone at 24, 72 hours or in combination with simvastatin at 24 hours (Table 3).

Regarding the combination therapy, administration of simvastatin and methyl-prednisolone as well as administration of rosuvastatin and methyl-prednisolone to rats with ALI caused significant reduction in the mean level of lung proteins at 72 hours and at the end of the experiment with the 1st combination and at 24 hours, 72 hours and at the end of the experiment with the 2nd combination when compared to the untreated model (Table 3).

The percentages of reduction was 2.5%, 43.5%, and 53.6% with simvastatin and methylprednisolone and 18.3%, 44.6%, and 53% with rosuvastatin and methylprednisolone respectively.

It was noticed that simvastatin and methylprednisolone combination therapy produced significant decrease of the percentage reduction with methylprednisolone was 19.2% at 72 hours and 50% at the end of the experiment.

The mean level of lung proteins was significantly reduced more with rosuvastatin than either simvastatin or methylprednisolone therapy after 72 hours, and than simvastatin at the end of the experiment (Table 3).

It was noticed that the reduction in the level of this parameter was more significant with methylprednisolone than simvastatin at the end of the experiment (Table 3).

Table (3): Effects of the tested drugs of the study on the mean (±SD) levels of protein (g/g) in lung tissue obtained from rats with ALI.

<table>
<thead>
<tr>
<th>Groups</th>
<th>–24 hours after ALI induction</th>
<th>–72 hours after ALI induction</th>
<th>–7 days after ALI induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rats (group 1).</td>
<td>0.50±0.07</td>
<td>0.60±0.07</td>
<td>0.50±0.05</td>
</tr>
<tr>
<td>Model of ALI (group 2).</td>
<td>1.20±0.14a</td>
<td>1.410±0.09a</td>
<td>1.64±0.15a</td>
</tr>
<tr>
<td>ALI + saline oral (0.1ml/day) (group 3).</td>
<td>1.40±0.42a</td>
<td>1.52±0.10a</td>
<td>1.70±0.24a</td>
</tr>
<tr>
<td>ALI + saline IV (0.1ml/day) (group 4).</td>
<td>1.40±0.18a</td>
<td>1.48±0.08a</td>
<td>1.68±0.10a</td>
</tr>
<tr>
<td>ALI + simvastatin (15mg/kg/day) (group 5).</td>
<td>1.20±0.18</td>
<td>1.00±0.16b</td>
<td>0.95±0.03b</td>
</tr>
<tr>
<td>ALI + rosuvastatin (15mg/kg/day) (group 6).</td>
<td>1.18±0.05</td>
<td>0.83±0.04b</td>
<td>0.76±0.07b</td>
</tr>
<tr>
<td>ALI + methylprednisolone (2mg/kg/day) (group 7).</td>
<td>1.30±0.17</td>
<td>1.14±0.16b</td>
<td>0.82±0.08b</td>
</tr>
<tr>
<td>ALI + combined simvastatin + methylprednisolone (group 8).</td>
<td>1.17±0.10</td>
<td>0.79±0.06b</td>
<td>0.77±0.05b</td>
</tr>
<tr>
<td>ALI + combined rosuvastatin + methylprednisolone (group 9).</td>
<td>0.98±0.06bcdef</td>
<td>0.78±0.02bcedef</td>
<td>0.77±0.05bc</td>
</tr>
</tbody>
</table>

The data expressed as (mean ± S.D) (n=6).
a: Significance as compared to normal (p<0.05).
b: Significance as compared to untreated model (p<0.05).
c: Significance as compared to simvastatin alone (p<0.05).
d: Significance as compared to rosuvastatin alone (p<0.05).
e: Significance as compared to methylprednisolone alone (p<0.05).
f: Significance as compared to combined simvastatin and methylprednisolone (p<0.05).
Results of the histopathological study:

The results were expressed as the mean (±SD) of the pathological score of lung tissues.

The mean (±SD) pathological score of lung tissue obtained from normal rats were 1.50±0.55 after 24 hours, 1.33±0.52 and 1.33±0.52, at 72 hours and at the end of the experiment, respectively (Table 4, Fig. 1).

Induction of ALI in rats (group 2) increased significantly the mean pathological score all through the experimental periods compared to normal rats (group 1) (Table 4, Fig. 2).

Oral or IV administration of saline (control groups 3 and 4) for rats with ALI did not produce significant change of the elevated pathological score in the model (group 2) all through the experiment (Table 4).

Compared to the untreated model group, the pathological score (mean± SD) was significantly decreased in the simvastatin treated group (Table 4, Fig. 3), the rosuvastatin treated group (Table 4, Fig. 4), and the methylprednisolone treated group (Table 4, Fig. 5) at all time periods. The percentage reduction of the pathological score after 24 hours, 72 hours and at the end of the experiment were 47.1%, 45% and 26.6%, for simvastatin; 48.8%, 52.6% and 39.3%, for rosuvastatin and 63%, 59.7% and 41.33%, for methylprednisolone respectively when compared to the untreated model group.

The improvement in the pathological score was significantly more apparent with rosuvastatin therapy than simvastatin after 72 hours and at the end of the experiment (Table 4).

The improvement in the pathological score was significantly more apparent in the methylprednisolone treated group when compared to simvastatin treated group and rosuvastatin at 24 hours after induction of ALI (Table 4).

Combined simvastatin and methyl-prednisolone treatment produced significant reduction in the mean pathological score when compared to the untreated ALI model at all time periods (Table 4, Fig. 6). The percentages of improvement were 61.3% at 24 hours, 68.7% at 72 hours and 56% at 7 days after induction of ALI.

Moreover the amelioration of the pathological score observed with this combination therapy was significantly more apparent than simvastatin or rosuvastatin therapy alone all through the experiment, and than methylprednisolone alone at 72 hours and at the end of the experiment (Table 4).

Rosuvastatin and methylprednisolone combination therapy significantly reduced the mean pathological score when compared to the untreated ALI model group 2 at all time periods (Table 4, Fig. 7). The percentages of reduction were 65.9%, 69.1 %, and 70% at 24, 72 hours and 7 days after induction of LPS induced ALI. Significal amelioration was more apparent with the latter combination therapy than simvastatin or rosuvastatin alone all through the experimental periods or methylprednisolone alone at 72 hours and 7 days or in combination with simvastatin at 7 days after induction of ALI (Table 4).

### Table (4): Effects of the tested drugs on the mean (±SD) lung tissue pathological score in different studied groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pathological score mean (±SD)</th>
<th>−24 hours after ALI induction</th>
<th>−72 hours after ALI induction</th>
<th>−7 days after ALI induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rats.</td>
<td>1.50±0.55</td>
<td>1.33±0.52</td>
<td>1.33±0.52</td>
<td></td>
</tr>
<tr>
<td>Model of ALI.</td>
<td>17.66±0.81a</td>
<td>21.17±2.32a</td>
<td>15.00±2.83a</td>
<td></td>
</tr>
<tr>
<td>ALI + saline oral (1ml/day)</td>
<td>18.00±0.03a</td>
<td>23.00±3.04a</td>
<td>16±2.73a</td>
<td></td>
</tr>
<tr>
<td>ALI + saline IV (0.1ml/day)</td>
<td>16.20±0.76a</td>
<td>20.16±2.73a</td>
<td>16.33±2.43a</td>
<td></td>
</tr>
<tr>
<td>ALI + simvastatin (15mg/kg/day)</td>
<td>9.33±1.03b</td>
<td>11.66±0.52b</td>
<td>11.00±1.10b</td>
<td></td>
</tr>
<tr>
<td>ALI + rosuvastatin (15mg/kg/day)</td>
<td>9.00±2.097b</td>
<td>10.00±0.89bc</td>
<td>9.166±1.94bc</td>
<td></td>
</tr>
<tr>
<td>ALI + methylprednisolone (2mg/kg/day)</td>
<td>6.50±1.048bedc</td>
<td>8.50±1.378bcde</td>
<td>8.83±2.48bcede</td>
<td></td>
</tr>
<tr>
<td>ALI + combined simvastatin + methylprednisolone</td>
<td>6.83±0.983bedc</td>
<td>6.66±1.7bbedc</td>
<td>6.66±0.61bdede</td>
<td></td>
</tr>
<tr>
<td>ALI + combined rosuvastatin + methylprednisolone</td>
<td>6.00±1.26bedc</td>
<td>5.50±1.6bdede</td>
<td>4.50±0.5bbedf</td>
<td></td>
</tr>
</tbody>
</table>

The data expressed as (mean ± S.D) (n=6).

a: Significance as compared to normal (p<0.05).
b: Significance as compared to untreated model (p<0.05).
c: Significance as compared to simvastatin alone (p<0.05).
d: Significance as compared to rosuvastatin alone (p<0.05).
e: Significance as compared to methylprednisolone alone (p<0.05).
f: Significance as compared to combined simvastatin and methylprednisolone (p<0.05).
Fig. (1): Normal structure of lung tissue (400).

Fig. (2): Massive intra-alveolar, perivascular infiltration of neutrophils in the ALI model at 72 hours and at the end of the experiment (400).

Fig. (3): Simvastatin treatment of ALI showing moderate perivascular oedeme and moderate neutrophilic infiltration at 72 hours and at the end of the experiment.

Fig. (4): Rosuvastatin treatment of ALI showing moderate perivascular neutrophilic infiltration at 72 hours and at the end of the experiment.
Fig. (5): Methylprednisolone treatment of ALI showing mild congestion and neutrophilic infiltration at 72 hours and at the end of the experiment.

Fig. (6): Combined simvastatin and methylprednisolone treatment of ALI showing mild congestion and leucocytic infiltration at 72 hours and at the end of the experiment.

Fig. (7): Combined rosuvastatin and methylprednisolone of ALI showing very mild perivascular leucocytic infiltration and congestion at 72 hours and at the end of the experiment.

Discussion

Statins were introduced as cholesterol lowering agents and considerable research has been focused on them. The present study was designed to focus on their effect either alone or in combination with corticosteroid in modulation of ALI.

An accepted standard for reliably estimating neutrophil infiltration into inflamed tissues is Myeloperoxidase (MPO) activity [13]. In the current study simvastatin was found to decrease significantly myeloperoxidase (as a marker of neutrophil extravasation) and malondialdehyde (as a marker of oxidative stress) levels and histopathological
changes in lung tissue in ALI at 24 hours, 72 hours and at the end of the experiment when compared to the untreated model of ALI. It also decreased significantly total lung protein concentration (as a marker of endothelial disruption) at 72 hours and at the end of the study when compared to the untreated model of ALI.

These results were in agreement with Sun et al., [14]. In ALI model induced by Ischaemia-reperfusion (I/R) of the hind limbs, rats were pretreated with 5 or 10mg/kg per day simvastatin. Lung tissue myeloperoxidase, malondialdehyde, neutrophil count and lung injury scores in both simvastatin groups were significantly lower than in the I/R group; 10mg/kg per day simvastatin significantly reduced lung water content although 5mg/kg per day did not. Expression of Haem Oxygenase-1 (HO-1) protein in lung tissue was significantly greater in the simvastatin groups than in the I/R group. Haem oxygenases have emerged as proteins with antioxidant, cytoprotective, neutrotransmitter and anti-inflammatory functions. The authors have indicated that inducing HO-1 expression in lung tissues with simvastatin might be a promising therapeutic approach in treating ALI. Moreover Grommes et al., [15] were also in agreement with this. Mice were exposed to aerosolized LPS for 30min to induce a model of ALI. They found that neutrophil depletion abolished permeability increases, elastase accumulation, and structural changes induced by LPS. Furthermore, simvastatin treatment reduced LPS-induced permeability changes to a degree similar to what was observed by neutrophil depletion suggesting that simvastatin primarily acts via modulating neutrophil function. Finally, simvastatin treatment blocked accumulation of neutrophil elastase in the bronchoalveolar lavage fluid. Regarding in-vitro study, they found that pretreatment of isolated human neutrophils with simvastatin for 3 hours abolished neutrophil adhesion indicating a direct effect on neutrophil adhesive functions, and degranulation. In addition pretreatment of neutrophils with simvastatin for 3 hours abolished reactive oxygen species formation.

Data suggested that Toll-Like Receptor 4 (TLR4) played a particular role in the regulation of neutrophil life span [16]. Studies have shown that lipopolysaccharide challenge upregulates TLR4 expression [17]. One of the major pathways activated by TLR4 is the activation of the Nuclear Factor-B (NF-B), which acts as a master switch for inflammation, regulating the transcription of many genes that encode proteins involved in immunity and inflammation [18]. Shao et al., [19] showed that simvastatin caused down regulation of TLR4 and NF-__B expressions and suppressed lung inflammatory response in a rat cardiopulmonary bypass model of ALI.

In a study conducted by Jacobson et al., [20], mice were treated with simvastatin 24h before and again concomitantly with intratracheally administered LPS. Simvastatin decreased BAL albumin (50% reduction) and evans blue albumin dye extravasation into lung tissue (100%). These findings are consistent with endothelial barrier protection as a result of simvastatin treatment. It is suggested that this barrier protection effect of simvastatin could possibly be caused by decreased geranylgeranylation and subsequent cytoskeletal rearrangement [21].

It could also be due to inhibition of the RhoA/Rho kinase pathway by statins with consecutive reduction of endothelial myosin light chain phosphorylation [22], stabilization of endothelial junctions by polymerization of cortical actin, as well as downregulation of endothelial caldesmon. In addition Chen and his colleagues [23] found that simvastatin induced integrin $\beta$4 expression, which could have an effect on endothelial barrier stability and signaling.

In contrast Shyamsundar and his collaegues [24] stated that there were no differences in mean (SD) BALF protein concentrations between placebo-treated or simvastatin treated subjects in healthy volunteer after LPS inhalation.

The results of the present work concerning myeloperoxidase were in contrast with the results of Altintas et al., [25]. The myeloperoxidase levels were higher in both the endotoxin and oleic acid induced murine models of ALI groups, as compared to the control group. Pretreatment with simvastatin did not significantly change the myeloperoxidase level in the endotoxin group, but it did decrease the myeloperoxidase level in the oleic acid group. The lack of significant difference in the endotoxin group can be explained by the fact that endotoxin caused an explosive inflammatory response that could not be totally controlled with simvastatin alone.

In the current study rosuvastatin treatment resulted in significant decrease in myeloperoxidase and improvement of lung histopathology in ALI at 24, 72 hours and at the end of the experiment when compared to the untreated model.
The results of the present work were in agreement with Kumar et al., [26] who evaluated the anti-inflammatory effect of rosuvastatin in models of acute and chronic inflammation. Rosuvastatin blocked the influx of PMN leukocytes into the rat paw and showed inhibition of second phase of carrageenan-induced rat paw edema, suggesting the inhibition of prostaglandins release. These data indicate that rosuvastatin is able to significantly attenuate the inflammatory process, thus preventing leukocyte infiltration, which might provoke subsequent tissue injury. In the chronic model of inflammation, cotton pellet-induced granuloma formation, the serum level of Alkaline Phosphatase (ALP) was found to increase in the control group but was significantly decreased by rosuvastatin at 20mg/kg. Marked decrease in serum enzyme levels of Alkaline Phosphatase (ALP) indicates that there was reduction in necrosis of cells, which may be mediated by inhibiting the release of inflammatory mediators that are responsible for tissue destruction and cell necrosis.

The current results are also in agreement with Dolkart et al., [27] a model of blunt chest injury in rat was employed. Lung contusion, is a leading risk factor for development of Acute Lung Injury (ALI) and acute respiratory distress syndrome. Histopathologic examination of lung tissue showed that rosuvastatin decreased significantly neutrophilic infiltration, hemmorhage and eadema. Rosuvastatin increased the expression of inducible nitric oxide (iNOS), COX-2, heme oxygenase-1 (HO-1), and prostaglandin E2 (PGE-2) in the bronchoalveolar lavage fluid of the rat contused lungs. The enhanced activity of iNOS, COX-2, and HO-1 in the lung may reflect the advent of protective processes that took place in the contused lung.

In the present study rosuvastatin was shown to decrease total lung protein as an indicator of lung edema significantly after 72 hours and at the end of the experiment when compared to the untreated model of ALI.

This result was in agreement with Matsuo et al., [28] who investigated the protective effect of rosuvastatin against I-R injury in lungs. The authors showed that rosuvastatin decreased IR lung injury, wet-to-dry ratio (as a measure of lung edema), and macrophage infiltration. Endothelial Nitric Oxide Synthase (eNOS) and phospho-eNOS were down-regulated by IR, which was blocked by rosuvastatin. The authors concluded that a single-dose of rosuvastatin decreased IR injury in lungs via two anti-inflammatory mechanisms: Preserving eNOS function and inhibiting macrophage infiltration.

In the present study rosuvastatin was shown to decrease significantly lung tissue malondialdehyde in ALI model at 24 hours, 72 hours and at the end of the experiment.

Jiang et al., [29] investigated the effects of rosuvastatin on ischemia-reperfusion injury in patients with acute coronary syndrome after Percutaneous Coronary Intervention (PCI). Malondialdehyde (MDA) is a marker of lipid peroxidation, which is detrimental to cellular function. The authors showed that the level of serum MDA was lower, in patients treated with rosuvastatin. This suggests that rosuvastatin could reduce oxidative stress.

The antioxidant effect of rosuvastatin does not depend on its cholesterol-decreasing properties. However, the inhibition of Nicotinamide Adenine Dinucleotide Phosphate Hydrogen (NADPH) oxidase activity with the inhibition of HMG-CoA reductase suggest that the mevalonate pathway plays a role in the antioxidant activity of rosuvastatin [30]. NADPH oxidase plays a role in the formation of the superoxide radical [31]. By inhibiting this molecule, rosuvastatin may decrease the amount of superoxide radical; as a result, SOD may be insufficiently consumed, and consequently, its level may be increased [32].

The present results were in contrast with Radi- gan et al., [33]. Lung injury was induced by either [H3N2] or [H1N1] influenza a virus. They found that rosuvastatin in a dose of 10mg/kg/day for three days prior to infection, did not inhibit influenza a viral replication in vitro and in vivo. Also rosuvastatin did not induce any differences in the percentage of inflammatory cells, proteins in BAL fluid and histologic severity of the lung and in that model of lung injury.

Methylprednisolone was chosen to be used in the current study because it has better concentration in the lungs than other corticosteroids due to its larger volume of distribution, longer mean residence time and greater retention in the epithelial lining fluid of the alveoli [34]. A relatively low dose of methylprednisolone (2mg/kg 3 i.v.), since this had been proved to be effective at avoiding lung mechanical changes in mild ALI and minimising alterations in tissue impedance in ALI [35].

In the present work treatment of LPS acute lung injury in rats with low dose of methylprednisolone (2mg/kg/day I.V) resulted in significant decrease in myeloperoxidase and improvement of
histopathology of the lungs at 24, 72 hours and at the end of the experiment.

Sevimli and his colleagues [36] studied the effect of methylprednisolone treatment on acid aspiration model of ALI in rabbits. Histopathologically methylprednisolone was effective in reducing lung damage, alveolar congestion, wall thickening, neutrophil infiltration and aggregation and hyaline membrane formation.

Moreover, in the present work methylprednisolone treatment of LPS-induced ALI resulted into significant decrease of lung proteins at 72 hours and at the end of the experiment. This was in agreement with Kunihiko et al., [37]. They concluded that methylprednisolone significantly reduced the paraquat-induced increase in pulmonary vascular permeability and in proctasin production.

In contrast to the results of the current study Müller et al., [38] conducted a study where a model of Transfusion Related Acute Lung Injury (TRALI) mice were primed with lipopolysaccharide. Administration of methylprednisolone did not decrease wet-to-dry ratio or BALF protein levels. Lung injury scores did not differ between treated and untreated mice. The lack of effect of methylprednisolone on lung injury might possibly be due to the fact that inflammatory damage in TRALI is mediated via pathways which are not affected by the administration of steroids.

In the current study methylprednisolone treatment was associated with significant decrease lung malondialdehyde (a marker of lipid peroxidation) at 24, 72 hours and at the end of the experiment.

This result is in agreement with Kuleci et al., [39]. In patients with stable COPD, methylprednisolone was given. Plasma MDA levels were significantly lowered only in methylprednisolone treated patients.

In contrast, Araujo et al., [40] found that systemic administration of methylprednisolone had no influence on parameters related to oxidative stress in lungs of brain dead donor rats undergoing pulmonary transplantation.

In the current study rosuvastatin was more effective in treatment of ALI than simvastatin in all parameter measured.

Ferreira et al., [41] investigated the effects of four different statins on acute lung inflammation induced by Cigarette Smoke (CS). Mice exposed to CS were grouped and treated with vehicle (i.p.), atorvastatin (10mg/kg), pravastatin (10mg/kg), rosuvastatin (5mg/kg), or simvastatin (20mg/kg). Treatment with statins differentially improved the pulmonary response when compared to the CS group. Rosuvastatin demonstrated the best anti-inflammatory effect, whereas simvastatin (in contrast to the present work) demonstrated the best antioxidant response.

Seker et al., [42] reported that rosuvastatin produced the most pronounced anti-epileptic effect in rats compared to simvastatin and atorvastatin. Rosuvastatin improved Blood-Brain Barrier (BBB) integrity, increased expression of endothelial Nitric Oxide Synthase (eNOS) mRNA and decreased expressions of pro-apoptotic p53, bax and caspase-3 mRNAs.

On the other hand, hypercholesterolemic subjects received simvastatin 40mg or simvastatin/ezetimibe 10/10mg or rosuvastatin 10mg daily for 12 weeks to compare their effects on markers of inflammation and oxidative stress. Moutzouri and his colleagues [43] stated that Simvastatin 40mg, simvastatin/ezetimibe 10/10mg and rosuvastatin 10mg significantly reduced 8-epiPGF2a, oxLDL and Lp-PLA2 activity and mass to a similar extent.

In the current study the combination therapy of methylprednisolone with either simvastatin or rosuvastatin showed more significant effects than each one alone on lung tissue levels of malondialdehyde, myeloperoxidase, lung tissue proteins and histopathological score.

Maneechotesuwan et al., [44] suggested that the combination of a statin and a corticosteroid therapy in chronic airway inflammatory disease in asthma could augment the Treg/Th17 cell ratio and thus more effectively suppress airway inflammation in asthma.

Simvastatin was shown to enhance corticosteroid-activated NFkB-induction of Indoleamine 2, 3-Dioxygenase (IDO) in macrophages by activating type I interferons. It also enhanced the effect of corticosteroid on IL-10 release. So statin enhanced the anti-inflammatory effect of an inhaled corticos- teroid in asthma [45].

The current study concluded that significant improvement and amelioration of ALI biochemical and pathological changes could be achieved by treatment either with statins alone or in combination with methylprednisolone. The improvement was more apparent with rosuvastatin and methylprednisolone combination therapy.
References


تأثر آدوية إستاتين مقارنة بالمركبات الاسترودية،
وتأثيرهما معا على إصابة الرئة الحاد في الجرذان: دراسة مقارنة

الهدف من هذا العمل هو تأثير آدوية إستاتين بالميثيل بريدنزيولون وحدة أو مجمعة آدوية إستاتين مع الميثيل بريدنزيولون في إصابة الرئة الحاد. إصابة الرئة تم إحداثها بواسطة وضع ليوبوليسكاريد الخاص بالآكل كولاي في الآفة.

تم استخدام 120 من الجرذان البيضاء. الدراسة مقسمة إلى المجموعة 1 الطبيعية، المجموعة 2 المعالجة بالليوبوليسكاريد، المجموعة 3 المعلجة بحلول البلح، المجموعة 4 المعالجة بالسيمفاستاتين، المجموعة 5 المعالجة برزيدنزيولون، المجموعة 6 المعالجة بالميتيل بريدنزيولون، المجموعة 7 المعالجة بالميتيل بريدنزيولون مع السيمفاستاتين، المجموعة 8 المعالجة بالميثيل بريدنزيولون مع الزوفاستاتين، المجموعة 9 المعالجة بالميتيل بريدنزيولون مع الزوفاستاتين.

 множج مزيج من الإستاتينات، البروتينات في تجربة محاكاة المجال الجريضية بروبيونات السيمفاستاتين، والزوفاستاتين، والزوفاستاتين، والزوفاستاتين، والزوفاستاتين.

نتائج الدراسة أوضحت الأثاث، وضع مادة ليوبوليسكاريد. الالتحاق مع زيادة كل المتغيرات المقابلة في الرئة (ميوريبيركسيدين)

العدول الإنتان، إنتاج البروتينات، ومعجب التغيرات الهيستويولوجية، العلاج بأي من الإستاتينات أو الميثيل بريدنزيولون نتج عنه نقص ذو دالة إحصائية في ميوريبيركسيدين، مالوندي أدين أدين، ميوريبيركسيدين، مالوندي أدين، ميوريبيركسيدين، مالوندي أدين، ميوريبيركسيدين، مالوندي أدين، ميوريبيركسيدين، مالوندي أدين، ميوريبيركسيدين، مالوندي أدين، ميوريبيركسيدين، مالوندي أدين، ميوريبيركسيدين، مالوندي أدين، ميوريبيركسيدين، مالوندي أدين، ميوريبيركسيدين، مالوندي أدين، ميوريبيركسيدين، مالوندي أدين، ميوريبيركسيدين، مالوندي أدين، ميوريبيركسيدين، مالوندي أدين، ميوريبيركسيدين، مالوندي أدين، ميوريبيركسيدين، مالوندي أدين، ميوريبيركسيدين، مالوندي أدين، ميوريبيركسيدين، مالوندي أدين، ميوريبيركسيدين، مالوندي أدين، ميوريبيركسيدين، مالوندي أدين، ميوريبيركسيدين، مالوندي أدين، ميوريبيركسيدين، مالوندي أدين، ميوريبيركسيدين، مالوندي أدين، ميوريبيركسيدين، مالوندي أدين، ميوريبيركسيدين، مالوندي أدين، ميوريبيركسيدين، مالوندي أدين، ميوريبيرксيدين، مالوندي أدين، ميوريبيركسيدين، مالوندي أدين، ميوريبيركسيدين، مالوندي أدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين، ميوريبيركسيدين, ميوريبيركسيدين

خلصت نتائج الدراسة إلى أن دواء الاستاتين مثوله أو متحد بالميثيل بريدنزيولون أظهر تحسن ذو دالة إحصائية في التغيرات الكيميائية أو الهيستويولوجية التي تحدث في مرض إصابة الرئة الحاد. إجتماع ميثيل بريدنزيولون مع الزوفاستاتين كان الأكثر فعالية.