A new agent for treatment of acute respiratory distress syndrome: thymoquinone. An experimental study in a rat model

Ahmet Feridun Isik*, Ismail Katib, Irfan Bayram, Hanefi Ozbek

*Thoracic Surgery Department, Medical School, Yuzuncu Yil University, Van, Turkey
**Anesthesiology and Reanimation Department, Medical School, Yuzuncu Yil University, Van, Turkey
**Pathology Department, Medical School, Yuzuncu Yil University, Van, Turkey
**Pharmacology Department, Medical School, Yuzuncu Yil University, Van, Turkey

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Abstract

Objective: Acute respiratory distress syndrome (ARDS) and acute lung injury (ALI) are important clinical problems in thoracic surgery and critical care medicine. Most of the treatment methods are still supportive. Thymoquinone has anti-inflammatory, spasmolytic, bronchodilator and antibacterial effects. We studied its effects on ALI/ARDS in a rat model.

Methods: ALI/ARDS was developed in 40 Sprague-Dawley male rats (200-250 g in weight) by intratracheal instillation of human gastric juice (pH 1.2). Rats were treated with mechanic ventilator for 3 h. There were five groups: Control group (n=11); Steroid group (n=10); Ethanol group (n=5); Thymoquinone group (n=9) and Thymoquinone + Steroid group (n=5). No instillation except gastric juice was applied in the first group. Thymoquinone was given in dosage of 6 mg/kg, metilprednisolone in dosage of 10 mg/kg, ethanol 0.75 ml/kg intraperitoneally (IP). Blood gas analysis and compliance measurement were done. At the end of the third hour, rats were sacrificed and their lungs were excised for histopathological examination.

Results: In the thymoquinone group, the ratio of arterial oxygen to the fraction of inspired oxygen (PO2/FiO2) was significantly better compared to the other groups (P=0.000–0.043). Static compliance measurements revealed higher values in thymoquinone and thymoquinone + steroid groups. Histopathological examinations showed that affected lung tissue is lower in groups 2 and 4 (P=0.000–0.027).

Conclusions: This study revealed that thymoquinone improved oxygenation while both thymoquinone and steroids protect lung tissue from hazardous effects of human gastric juice (pH 1.2) histopathologically.

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Keywords: ARDS; Acute lung injury; Treatment methods; Thymoquinone

1. Introduction

Despite recent advances in medical technology and critical care, overall mortality of ALI/ARDS remains approximately 30-70% and requires aggressive therapy to maintain adequate oxygenation and ventilation [1-4]. Low pulmonary compliance and progressive atelectasis after the establishment of lung injury result in an increased work of breathing and an inadequate pulmonary gas exchange [2-12]. There are different but still limited treatment options including mechanical ventilation techniques, nitric oxide (NO), surfactant therapy, partial liquid ventilation, steroids, sodium nitroprusside (SNP), perfluorocarbons and extra-corporal CO2 removing [4,6-9,13,14-18].

Thymoquinone, whose chemical structure is C10H12O2, is a substance obtained from volatile oil of Nigella sativa [www.sigma-aldrich.com]. Many authors studied its cardiovascular, respiratory and anti-inflammatory effects [19-24]. It inhibits cyclooxygenase and 5-lipoxygenase pathways of arachidonate metabolism [22]. The effect was demonstrated to be via the dose-dependent inhibition of the formation of thromboxane B2 and leukotriene B4. Gilani and co-workers showed its bronchodilator effect [19]. Türkdoğan and colleagues have studied the effect of thymoquinone in carbon tetrachloride (CCL4) induced hepatotoxicity. They have found that it significantly protected liver from the hazardous effects of CCL4 and prevented fibrosis of liver in rats [20].

In view of these experimental studies, we aimed to investigate the effects of thymoquinone on ALI/ARDS, which can be defined as a rapid development of pulmonary fibrosis. We did not come across with thymoquinone usage or experimental study on ALI/ARDS in the literature.

2. Material and methods

The experimental protocols used in this study were approved by the Medical School Ethic Committee of Yuzuncu
Yil University at Van and based on European Convention on Animal Care.

2.1. Experimental animals

Male Sprague-Dawley rats (200-250 g) bred in the Animal House, Yuzuncu Yil University, were fed on standard rat diet and water ad libitum.

2.2. Chemical model

Thymoquinone (Sigma-274666), metilprednisolone (10 mg/kg), ethyl alcohol (ethanol) in 96% concentration were used. Thymoquinone was dissolved in ethanol in the ratio of 8 mg/ml and was given in dosage of 6 mg/kg as indicated in the literature [www.sigma-aldrich.com] [21,23,24]. Ethanol (0.75 ml/kg) was used in another control group, because it was used to dissolve thymoquinone. All chemicals were given intraperitoneally (IP).

2.3. Technical and mechanical equipments

For invasive pressure and heart rate monitoring, Petas, KMA-625R model monitor (Ankara, Turkey); for mechanical ventilation, Biomed Devices MVP-10 pediatric respirator (Boston, USA) and for blood gas analysis, Nova Biomedical-Phox Stat Profile (Massachusetts, USA) model devices were used.

2.4. Experimental study model

We modified the format of North America–Europe Consensus Congress (NAECC) criteria for ALI/ARDS and lung injury score scale defined by Murray (Table 1). Instead of chest X-ray score, we preferred to evaluate the histopathological findings.

Rats were classified into five groups: Control group, n = 11; Steroid group, n = 10; Ethanol group, n = 5; Thymoquinone group, n = 9 and Thymoquinone-steroid group, n = 5.

Ketamin was given IP in 25 mg/kg dosage for anesthesia. Tracheotomy was performed with vertical cervical incision. After tracheotomy, 1 mg/kg IP norcuron was given for muscle relaxation in order to obtain 5 ml tidal volume and ventilation pressures for initial value were recorded. Because of general anesthesia with muscle relaxation, static compliance was calculated according to formula Vt (ml/kg)/pressure (cmH2O) [12]. At the beginning of deep anesthesia and at the end of the third hour, the lungs were inflated with air through the tracheotomy catheter from 1 cmH2O pressure up to 30 cmH2O in order to measure compliance. We gave nothing except HGJ to the control group. Steroid group received metilprednisolone (10 mg/kg), ethanol group (ethanol, 0.75 ml/kg), thymoquinone group (thymoquinone, 6 mg/kg), thymoquinone-steroid group (thymoquinone, 6 mg/kg; and metilprednisolone, 10 mg/kg) at the fifth minute after HGJ instillation. Following instillation of HGJ, blood gas analysis was done and arterial pressure values were recorded at the fifth minute, first, second and third hour. At the end of the third hour, rats were sacrificed with the overdose of ketamin and lungs were excised after last measurements. The materials were fixed in 10% neutral-buffered formalin and embedded in paraffin. Four-micrometer-thick sections were cut and stained with hematoxyline and eosin.

Histopathological examination was done at times 5, 10, 20 and 40 magnifications in 1 cm² area of stained lung tissue. We searched thoroughly by microscopic examination for alveolar destruction, interstitial oedema, intra-alveolar hemorrhagia, destruction of alveolo-capillary membrane, migration of neutrophil leukocyte, hyaline membrane and intra-arterial thrombus. Existence of at least three of these findings near alveolar destruction was accepted as essential for histopathological scoring scale that was generated according to the affected area of lungs to evaluate if any significant difference occurred between the groups: 10% (1 point), 20% (2 points), 30% (3 points), 40% (4 points), 50% (5 points), 60% (6 points), 70% (7 points), 80% (8 points) and over 80% (9 points).

2.5. Statistical analysis

Blood gas and compliance analyses were done by one-way ANOVA, post hoc Tukey-HSD (Tukey’s Honesty Significant Difference) tests, whereas histopathological data were analyzed by two-tailed chi-square test in all groups. Data were presented as mean ±SE of the mean.

3. Results

Hemodynamic data were normal during the 3-h period (mean arterial pressure was 94.31 ± 6.02 mmHg), except three animals that were excluded from the study during statistical analysis because two rats in control group and one rat in ethanol group died before the third hour due to deep hypoxemia and acidosis.

Initial pressures were same in all animals (10 cmH2O). At the end of third hour, mean compliance with standard error was 2.39 ± 0.2205 in control group; 2.4 ± 0.2108 in steroid group; 2.5 ± 0.00 in ethanol group; 2.33 ± 0.25 in thymoquinone group and 2.3 ± 0.2739 in thymoquinone-steroid group. Last compliance values were significantly high in group 4 (P = 0.000-0.002) (Table 2).

Table 1

<table>
<thead>
<tr>
<th>Score</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy lung tissue in histopathological examination (%)</td>
<td>&gt;80</td>
<td>60-80</td>
<td>40-60</td>
<td>20-40</td>
<td>&lt;20</td>
</tr>
<tr>
<td>Hypoxemia score (PO2/FIO2)</td>
<td>&gt;300</td>
<td>225-229</td>
<td>175-224</td>
<td>100-174</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Compliance (%)</td>
<td>&gt;80</td>
<td>60-80</td>
<td>40-59</td>
<td>20-39</td>
<td>&lt;20</td>
</tr>
</tbody>
</table>

We modified NAECC and Murray scoring scale. Instead of chest X-ray, histopathological findings were used. Evaluation: 0 score, no injury; 1-3, mild changes; 4-6, moderate changes; 7-9, severe changes.
Changes in initial to last and instillation to last PO2/FiO2 values were compared and found that there was significant difference between group 4 and the other control groups ($31.08 \pm 10.3235$, $P = 0.004–0.036$) (Table 3). Comparisons of partial CO2 pressures and pH values revealed no significant difference.

In histopathological examination, it was observed that ALI/ARDS occurred in all groups, but affected lung area was significantly less in thymoquinone group compared with control, ethanol and thymoquinone + steroid groups ($P = 0.000–0.002$; $P < 0.05$) (Figs. 1 and 2). However, it was found that the effect of the steroid on histological evidence was meaningful ($P = 0.000–0.027$; $P < 0.05$) (Fig. 3) (Tables 4 and 5).

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Mean arterial pressure (mmHg)</th>
<th>Initial plato pressure (cmH2O)</th>
<th>Third hour plato pressure (cmH2O)</th>
<th>Initial compliance (ml/kg per cmH2O)</th>
<th>Third hour compliance (ml/kg per cmH2O)</th>
<th>Percent changes between third hour and initial compliance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>87.96</td>
<td>10</td>
<td>23.89</td>
<td>2.39 ± 0.2205</td>
<td>1.018 ± 0.1805</td>
<td>−57</td>
</tr>
<tr>
<td>Steroid group</td>
<td>94.17</td>
<td>10</td>
<td>19.5</td>
<td>2.4 ± 0.2108</td>
<td>1.302 ± 0.3475</td>
<td>−46.12</td>
</tr>
<tr>
<td>Ethanol group</td>
<td>92.08</td>
<td>10</td>
<td>27.5</td>
<td>2.5 ± 0.00</td>
<td>0.932 ± 0.9311</td>
<td>−62.72</td>
</tr>
<tr>
<td>Thymoquinone group</td>
<td>91.67</td>
<td>10</td>
<td>13</td>
<td>2.33 ± 0.2500</td>
<td>1.8238 ± 0.3512</td>
<td>−22.14</td>
</tr>
<tr>
<td>Thymoquinone + steroid group</td>
<td>105.67</td>
<td>10</td>
<td>15</td>
<td>2.3 ± 0.2739</td>
<td>1.5280 ± 0.4134</td>
<td>−33.56</td>
</tr>
</tbody>
</table>

Comparison of compliance values depending on plato ventilation pressures. There was significant difference between thymoquinone group and the others ($P = 0.000-0.002$; $P < 0.05$) [One way ANOVA and Tukey’s HSD test]. Plato ventilation pressures in thymoquinone group were lower; hence, higher compliance values occurred.

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Initial PO2/FiO2</th>
<th>Last PO2/FiO2</th>
<th>% Change</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>323.31 ± 21.61</td>
<td>116.29 ± 47.32</td>
<td>−64.69 ± 12.10</td>
<td>0.004</td>
</tr>
<tr>
<td>Steroid group</td>
<td>342.97 ± 53.46</td>
<td>172.97 ± 27.21</td>
<td>−48.27 ± 12.55</td>
<td>0.036</td>
</tr>
<tr>
<td>Ethanol group</td>
<td>427.98 ± 33.44</td>
<td>150.83 ± 45.96</td>
<td>−64.59 ± 11.74</td>
<td>0.004</td>
</tr>
<tr>
<td>Thymoquinone group</td>
<td>394.89 ± 91.13</td>
<td>257.60 ± 101.79</td>
<td>−31.08 ± 30.97</td>
<td>0.003</td>
</tr>
<tr>
<td>Thymoquinone + steroid group</td>
<td>402.06 ± 46.01</td>
<td>181.96 ± 77.01</td>
<td>−48.13 ± 6.03</td>
<td>0.003</td>
</tr>
</tbody>
</table>

It was found that oxygenation was significantly better in thymoquinone group when PO2/FiO2 values compared ($P < 0.05$) [One way ANOVA and Tukey’s HSD test].

### 4. Discussion

Thoracic surgeons, anesthesiologists and pulmonary clinicians are often faced with management problems of ALI/ARDS which are unexpected circumstances and needs immediate treatment [1-4]. Its diagnostic criteria have been well defined as follows: acute starting, bilateral lung infiltrates, PO2/FiO2 $<200$ and decrease in compliance more than 30%. The essential phenomenon is alveolar destruction with increased permeability of alveolo-capillary membrane. In thoracic surgery, pulmonary resections and trauma are the most common causes of ALI/ARDS. Currently, the reported mortality rates of ALI/ARDS following pulmonary resection range from 40 to 72.5% [1-4]. Ruffini et al. have reported mortality rates in 1221 patients who underwent pulmonary resection 40% for ALI and 59% for ARDS [1]. In this series, overall rate of ALI/ARDS was 2.2%. Kutlu and colleagues have found ALI/ARDS in a rate of 3.9% in their series and mortality rate was 72.5% [2]. Other causes of ALI/ARDS are prolonged high concentration O2 therapy, mechanical ventilation, sepsis, hyper blood transfusion and aspiration pneumonia [10,12,16-18]. Especially, aspiration...
pneumonia is a common cause in daily life today. Tseng et al. have reported 4.2% rate of ALI/ARDS among patients who attended to their clinic with corrosive acid ingestion [10]. They emphasize that pH of gastric juice determines the severity of lung injury.

Whatever the underlying process, specific therapy has been unavailable because of our lack of understanding of what triggers the problem and what mediates the injury to the lung that leads to the clinical syndrome. Treatment options are limited and most of them are supportive. Mechanical ventilation, NO therapy, nitroprusside, steroids, surfactant, partial liquid ventilation and extra-corporal CO2 removing are essential modalities [4–9,12–14]. We can say that pH of gastric juice must be major purpose in patients with ARDS. However, main purpose in treatment of ALI/ARDS must provide tissue healing beyond oxygenation. Treatment methods used in ALI/ARDS may be good procedures for decreasing morbidity of mechanic ventilation, but primary injury or disease should be treated for a real recovery. Therefore, other treatment methods should be investigated.

Many authors have researched thymoquinone extracted from Nigella sativa, and found its important effects in living organisms. Its bronchodilator, spasmyloytic, anti-inflammatory and antibacterial activities have been define and well known [19-21,22-24]. In an experimental study, Türkdoğan and his colleagues have showed that thymoquinone protected rat liver from toxicity of CCl4 [20]. In histopathological examination, liver fibrosis was not observed in rats, which were fed with thymoquinone. In the view of these data, we hypothesized that it can be effective in ALI/ARDS, which can be defined as rapid development of pulmonary fibrosis. The results of our study suggest that thymoquinone improves oxygenation but cannot decrease CO2. We speculated this effect because of lower pressures in thymoquinone group compared to the control groups. Mean compliance decreased 22% in thymoquinone group, while it decreased 50-55% in the other groups. Therefore, other groups needed higher pressures. This difference might be the cause of insufficient ventilation for CO2 removing in the thymoquinone group.

We can claim that thymoquinone improves oxygenation with bronchodilatation and prevents tissue damage caused by human gastric juice instillation. Histopathological findings were significantly different in thymoquinone group. Affected lung area was approximately 50% in thymoquinone group, whereas it was 84% in control group, 72.5% in ethanol group and 76% in thymoquinone + steroid group. However, the ratio of ALI/ARDS findings in steroid group was 57%. In fact, intra-alveolar hemorrhagia, neutrophil migration, interstitial edema and hyaline membrane were less in group 4 even in affected areas. Nevertheless, we cannot generate a scoring scale for this comment. In microscopic examination, it was easily seen that even affected sections were better compared to the control groups. In thymoquinone + steroid group, compliance analysis showed better results than the control and ethanol groups, but histopathological and blood gas findings were similar to those in the control and ethanol groups. This situation may be the result of bronchodilator effects of both steroid and thymoquinone. We comment that steroid and thymoquinone may have mutual antagonistic effects on tissue protection.

In conclusion, thymoquinone improves both oxygenation and compliance in human gastric acid (pH=1.2) induced ALI/ARDS. It provides this effect not only by bronchodilatation, but also by preventing heavy inflammatory response in the rat lung. In our opinion, thymoquinone can be applied in these patients. However, both experimental animal studies and clinical investigation on voluntary patients have to be performed for further exploration.

**Table 4**

<table>
<thead>
<tr>
<th></th>
<th>Affected lung area (%)</th>
<th>Healthy lung tissue (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>84</td>
<td>16</td>
<td>G2: 0.000; G4: 0.000</td>
</tr>
<tr>
<td>Steroid group</td>
<td>57</td>
<td>43</td>
<td>G1: 0.000; G3: 0.027; G5: 0.007</td>
</tr>
<tr>
<td>Ethanol group</td>
<td>73</td>
<td>27</td>
<td>G2: 0.027; G4: 0.001</td>
</tr>
<tr>
<td>Thymoquinone group</td>
<td>50</td>
<td>50</td>
<td>G1: 0.000; G3: 0.001; G5: 0.000</td>
</tr>
<tr>
<td>Thymoquinone + steroid group</td>
<td>76</td>
<td>24</td>
<td>G2: 0.007; G4: 0.000</td>
</tr>
</tbody>
</table>

According to scoring scale we modified, it was observed that thymoquinone and steroid prevented heavy inflammatory response in rat lungs compared with other groups. Although there was no significant difference between the steroid and thymoquinone, tissue damage was less in thymoquinone group (P<0.05).

**Table 5**

<table>
<thead>
<tr>
<th></th>
<th>Healthy area score</th>
<th>Oxygenation</th>
<th>Compliance</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Steroid group</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Ethanol group</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Thymoquinone group</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Thymoquinone + steroid group</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>7</td>
</tr>
</tbody>
</table>

Comparison of total scores of groups revealed thymoquinone can be considered as a therapeutic method in human gastric juice induced ALI/ARDS.

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**References**


