THE EFFECT OF CEPAE EXTRACTUM-HEPARIN-ALLANTOIN IN THE PREVENTION OF PLEURAL ADHESIONS FOLLOWING THORACOTOMY IN RATS

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ABSTRACT

Aims: The aim of this study was to assess the effects of cepae extractum-heparin on the prevention of pleural adhesions following thoracotomy in rats.

Methods: 20 female Sprague-Dawley rats were used in this study. The rats were randomized into three groups. group 1 underwent only a thoracotomy. Animals in group 2 underwent thoracotomy with pleural abrasion, and animals in group 3 underwent thoracotomy with pleural abrasion and were treated with intrapleural application of cepae extractum-heparin-allantoin. The animals were sacrificed on the twenty-first day, and the adhesions were evaluated both microscopically and macroscopically.

Results: The mean adhesion severity scores for groups 1, 2, and 3 were 1.25±0.50, 2.6±1.41 and 1.25±0.46, respectively. While there were no significant differences in the length of adhesions between group 1 and 2 (p>0.05), the difference was significant between groups 1 and 3, and also between 2 and 3 (p<0.05 for both). Comparison of the groups with respect to the adhesion severity score showed that there were no statistically significant differences between the groups (p>0.05). The difference between the groups with respect to thicknesses of the parietal and visceral pleurae showed no significant difference (p>0.05).

Conclusion: The results showed that the use of cepae extractum-heparin-allantoin following thoracotomy decreased the pleural adhesions, however this decrease was not statistically significant.

Key words: Cepae extractum-heparin-allantoin, adhesion; pleura, rat.

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Introduction

Thoracotomy leads to pleural adhesions due to formation of scar tissues under the thoracotomy incision. When a re-thoracotomy is needed, pleural adhesions pose difficulties to thoracic surgeons during surgery. In thoracic surgery, the indications for re-thoracotomy include recurrent pneumothorax, chronic obstructive lung disease, lung infections, metachronous lung cancers, metastatic lung cancers, and kyphosis deformities fixed incrementally through an anterior spinal procedure\(^1,2\). The presence of pleural adhesions make it rather difficult to separate the lungs while gaining to access the pulmonary hilus. In addition, during lysis of adhesions, parenchymal lung hemorrhages and prolonged air leaks can occur. As a consequence the operative time is longer, therefore morbidity and mortality risks are increased\(^3\).

The routine use of adhesion-preventive agents in surgery may be clinically useful, however they may be expensive, affecting both the patients and the healthcare services by the heavy burden of the costs\(^4\).

Currently, physical barriers seem to be the most effective and convenient approach in prevention of adhesions. These materials separate the surfaces on the 3rd and the 5th days, which are the most critical days for postoperative adhesion formation.
There are two types of these barriers: the solid mechanical barriers (film and gel) and the liquid agents. These are divided into two groups, as absorbable and non-absorbable barriers\(^5,6\). There are only a few clinical and experimental studies about the prevention of adhesions in chest surgery. However in the literature there are various experimental animal studies about the use of adhesion preventive agents in thoracotomies. Karacam et al. searched the effectiveness of the use of polyethylene glycol to prevent pleural adhesions in rats\(^7\). Tanaka et al. demonstrated that absorbable membranes containing hyaluronate prevented the post-thoracotomy pleural adhesions in rats\(^8\). Getman et al. showed that in patients undergoing re-thoracotomies for any reason, adhesions did not occur with the application of Tachosil to control bleedings and to prevent air leakages in organ injuries. They also demonstrated the adhesion-preventive effect of Tachosil in an experimental rat study\(^2\). Takagi et al. demonstrated in their rat study that DL powder, seprafilm and intercede were highly effective in preventing postoperative pleural adhesions; however, DL powder application was easier in VATS\(^9\).

Izumu and colleagues compared gamma-glutamic acid (XL) and seprafilm (SEP) in preventing adhesions following thoracotomy in rats and they demonstrated that XL was more effective than SEP\(^10\). To our knowledge, there are no previous animal studies on the use of Capea extract-heparin-allantoin in post-thoracotomies for the prevention of adhesions. On the other hand, their uses for prevention of adhesions were reported in abdominal surgery, plastic-reconstructive surgery and neurosurgery in animal studies. Temiz et al. surveyed the effect of the Capea extract-heparin-allantoin on prevention of epidural fibrosis in a rat hemilaminectomy model and showed that it decreased the acute and chronic inflammation\(^11\).

Aysan et al. investigated the effect of Capea extractum-heparin-allantoin on preventing postoperative peritoneal adhesions in rats. They also demonstrated that a single dose of the drug did not prevent the postoperative peritoneal adhesions, but it decreased the severity\(^12\). Dogramaci et al. studied the effect of the Capea extract on peritendinous adhesions in an experimental survey in rabbits. They determined that perioperative use of a single dose of the Capea extract decreased the peri-tendinous adhesions\(^13\).

The aim of this experimental study was to evaluate the efficacy of Capea extractum-heparin-allantoin gel in the prevention of pleural adhesions following thoracotomy and pleural abrasion rats.

Materials and methods

This study was carried out after approval of the Istanbul University Animal Research and Ethics Committee, and was conducted under laboratory conditions by two surgeons. However, to prevent variations in handling adhesions only one surgeon performed surgical operations. Twenty Sprague-Dawley adult female rats weighing 200-300 grams and aged 3 months were used. All rats were fed by standard micro pellet feeds ad libitum. The rats were randomized into 3 groups as the thoracotomy only (group 1) (n=4), thoracotomy and pleural adhesion (group 2) (n=8), and thoracotomy with pleural adhesion followed by the application of Capea extract-allantoin-heparin (group 3) (n=8). The rats were anesthesized with intraperitoneal injections of 25 mg/kg ketamine and 10 mg/kg xylazine. Then a tracheostomy was opened in each animal, and intubated with a 10 G plastic cannula.

The rats were placed in the right decubitus position, the left lateral thoracic area was shaved and disinfected. The operations were performed under sterile conditions. In all 3 groups, an approximately 30 mm skin incision was made and a left posterolateral thoracotomy was performed from the 5th intercostal space (Fig. 1).

Figure 1: Macroscopic images of the rats (Left thoracotomy, 5\(^{th}\) intercostal space).

Only left sided thoracotomy was performed on the rats in group 1. In group 2, after left thoracotomy, an intrathoracic wall (including the visceral and the parietal pleura) abrasion was performed with a sterile gauze, and an adhesion model was created\(^14\). In group 3, following left thoracotomy, sterile gauze abrasion was made identical to group 2 and
then 2 cc of “cepae extract-allantoin-heparin” gel (Contractubex gel, Merz Pharma, Frankfurt, Germany) was administered to the left hemithoracic cavity, spreading it to the entire visceral and parietal pleurae by application with a injector. There was no physical contact with the visceral and parietal pleura except for the drug itself. After control of bleeding and air leak in all animals, the ribs were reapproximated using prolene 3/0 sutures, and the muscle, subcutis, and skin were repaired with vicryl 3/0 sutures. The rats were extubated after their spontaneous respiration returned to normal and all were placed in separate cages. None of the rats received pre- or postoperative antibiotherapy. Sufficient amount of water and food were placed into the cages. None of the rats exhibited signs of infection during the follow-up period. On the 21st day of the operation, the time when wound healing was considered to be complete and the adhesion preventive gel completely absorbed, all rats were sacrificed using a lethal dose of 135 mg/kg intraperitoneal sodium pentothal.

In the second step, sacrificed rats were evaluated through a left posterolateral thoracotomy from the 8th intercostal space and macroscopic adhesion severity scoring was performed. The longest adhesion at the first thoracotomy incision site was measured in millimeters. Then, the severity of adhesion scoring between the parietal pleura and the lung was measured. These were rated as: 1= no adhesion, 2= loose and could be abolished by blunt dissection, 3= moderate, some of them could be abolished by sharp dissection, 4= severe, all could be abolished by sharp dissection (Fig. 2). Adhesion severity scoring was performed by 2 separate researchers.

Following macroscopic evaluation of all groups, en bloc removal of the left thoracic wall was performed together with the left lung. This was fixed with 10% formaldehyde and sent for histopathological examination. Macroscopically, an en bloc sample was extracted from the side with the most severe changes on the parietal and visceral pleura. Also, 5 mm en bloc samples parallel to these areas were extracted. The extracted materials were decalcified with 10% formic acid. Following the routine tissue procedure, they were plunged into paraffin and cross sections 3-5 micrometers in size were prepared. The cross sections were dyed by Hematoxyline-Eosin (HE) and Masson-trichrome dyes. They were then evaluated under a light microscope (Olympus BX51, Tokyo, Japan). by pathologists who were blinded to the experimental rat groups. In the HE-dyed cross sections, the parietal and visceral pleural thicknesses were measured using an ocular linear micrometer (Olympus, Tokyo, Japan).

The measurements were made under x10 magnification, and chronic inflammation, increased vascularization and mesothelial hyperplasia in the parietal and visceral pleurae were assessed, also fibrosis in these structures was assessed semi-quantitatively with the Masson-trichrome (absent= 0, slight=1, moderate=2, severe=3) stain. The presence of foreign body reaction and myxoid degeneration were recorded.

The SPSS 15.0 program was used for the statistical analysis. The Fischer’s Exact Test, the Mann Whitney U Test, and the Kruskal Wallis Test were used for the comparison of parameters between the groups. In this study power calculation was not done. The significance level was considered as p value of <0.05.

Results

All three groups were evaluated macroscopically and microscopically following thoracotomy. The rats were evaluated for the length of adhesion, the adhesion severity score, pleural thickness, foreign body reaction, vascularization and fibrosis.

Development of adhesion was seen in one of the four rats in group 1, in 5 of the 8 rats in group 2, and in 2 of the 8 rats in group 3 (Table 1).

However, comparison between the groups showed no statistically significant difference with respect to the presence of adhesions between groups 1 and 3, or between group 1 and 2, or between group 2 and 3. (Fisher’s Exact Test, p>0.05).

While no statistically significant difference was observed in the adhesion length between group 1 and 2 or between group 1 and 3 (p>0.05), there

Figure 2: Macroscopic images of the rats after sacrifice (Adhesion image, group 3).

The Effect of Cepae extractum-Heparin-Allantoin in the Prevention of Pleural Adhesions Following Thoracotomy in Rats
was a statistically significant difference between groups 2 and 3 (Mann Whitney U test, p<0.05).

When three groups were compared between each other for the difference in adhesion length and adhesion severity score, there was no significant difference statistically between the groups (p>0.05) (Table 2). Also, when the three groups were compared regarding parietal and visceral thicknesses, there was no statistically significant difference between them (p>0.05) (Table 3). Likewise, in evaluation of the parietal and visceral thicknesses in all groups there were no statistically significant differences between group 2 and 3, between group 1 and 2, and between group 1 and 3 (Mann Whitney U test, p>0.05).

Only one rat in the control group and one rat in the study group had developed foreign body reaction. However, there was no statistically significant difference between groups 2 and 3 with regard to parietal and visceral pleural foreign body reaction (Fischer’s Exact Test, p>0.05).

All of the 8 rats in group 2, and 7 of the 8 rats in group 3 had developed parietal pleura foreign body reactions. There was no statistically significant difference between group 2 and 3 with respect to parietal pleura foreign body reaction, (Fischer’s Exact Test, p>0.05).

None of the rats in group 2 developed visceral pleura myxoid degeneration, while 6 of the rats in group 3 developed myxoid degeneration. When compared, there was no statistically significant difference between groups 2 and 3 with respect to visceral pleura myxoid degeneration (Fischer’s Exact Test, p>0.05).

Comparison between the groups with respect to the severity of visceral and parietal pleural vascularization and also chronic inflammation severity did not show a statistically significant difference (Kruskal Wallis T, p>0.05).

It was observed that 6 rats in group 2 and 8 rats in group 3 developed visceral mesothelial hyperplasia. Groups 2 and 3 did not have a statistically significant difference when compared for the development of visceral mesothelial hyperplasia. (Fisher’s Exact Test, p>0.05).

Parietal mesothelial hyperplasia had developed in 4 rats in group 2 and 5 rats in group 3, there was no significant difference between the two groups (Fisher’s Exact Test, p>0.05).

### Table 1: Comparison of adhesion severity scoring and adhesion length between the groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Adhesion length (mm)</th>
<th>Adhesion severity score*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>1</td>
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<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
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<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Group 2</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
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<td>8</td>
<td>9</td>
<td>4</td>
</tr>
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<td>1</td>
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<td>5</td>
<td>0.5</td>
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<td></td>
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<td>1</td>
<td>2</td>
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<td>7</td>
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<td>1</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

*Scoring of the adhesion severity between the parietal pleura and the lung: 1: no adhesion, 2: loose and could be abolished by blunt dissection, 3: moderate, some of them could be abolished by sharp dissection, 4: severe: all could be abolished by sharp dissection(14).

### Table 2: Statistical comparison of groups by mean adhesion length and mean adhesion severity score.

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Adhesion length (mm) (Mean ± Sd)</th>
<th>p*</th>
<th>Grade score (Mean ± Sd)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>4</td>
<td>0.75±1.50</td>
<td>0.083</td>
<td>1.25±0.50</td>
<td>0.084</td>
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<td>Group 2</td>
<td>8</td>
<td>4.00±3.66</td>
<td></td>
<td>2.62±1.41</td>
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</tr>
<tr>
<td>Group 3</td>
<td>8</td>
<td>0.19±0.57</td>
<td></td>
<td>1.25±0.46</td>
<td></td>
</tr>
</tbody>
</table>

*Kruskal Wallis Test.

### Table 3: Statistical comparison of groups by mean parietal pleura and visceral pleura thickness.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Parietal pleura thickness (100 Unit: 1 mm) (Mean ±Sd)</th>
<th>p*</th>
<th>Visceral pleura thickness (100 Unit: 1mm) (Mean ±Sd)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>4</td>
<td>25.25±20.41</td>
<td>0.064</td>
<td>13.50±10.21</td>
<td>0.284</td>
</tr>
<tr>
<td>Group 2</td>
<td>8</td>
<td>26.25±29.10</td>
<td></td>
<td>14.25±19.57</td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>8</td>
<td>21.25±10.25</td>
<td></td>
<td>30.50±12.65</td>
<td></td>
</tr>
</tbody>
</table>

*Kruskal Wallis Test
Discussion

Cepae extractum-heparin-allantoin is commonly used in the treatment of hypertrophic scars and keloids in clinical practice\textsuperscript{(15)}. Cepae extractum-heparin-allantoin is basically an onion extract. It contains only 50 U of sodium heparin and 1% allantoin. The onion extract is derived from the natural onion plant and is known as contractubex® in Europe and as mederma® in the USA. As all of these preparations contain 1% allantoin, while contractubex® also contains heparin. Heparin augments the anti-inflammatory effect of the onion extract and increases the collagen restoration\textsuperscript{(16)}. The mechanism of cepae extractum-heparin-allantoin efficiency shows variations in time. While cepae extractum-heparin-allantoin decreases the inflammation and fibroblastic proliferation seen in the early phase of wound healing, it decreases the tissue components (proteoglycans, collagen) in the later phase of wound healing\textsuperscript{(17)}. In a prospective multi-center clinical trial (38 centers, 859 patients), it was reported that cepae extractum-heparin-allantoin was more effective in the treatment of hypertrophic scars than topical corticosteroids\textsuperscript{(18)}.

Following pleural damage, growth factor and cytokines are released and the vascular permeability increases. Then, the fibrinogen forms fibrin in the extravascular space fluid, forming fibrotic bands. In the healing process, fibrotic bands are destroyed by fibrinolytics and the mesothelial layer is repaired. The fibrinolytic system can be inhibited by trauma, infection and hypoxia. Accumulation of fibroblasts with other cells and collagens lead to organization of bands, thus irreversible adhesions can be formed among the serosal surfaces and organs. Consequently, they can cause serious complications. The most common site of post operative adhesions is the abdomen which requires an urgent re-operation. The adhesion rate following laparotomies is approximately 90\%\textsuperscript{(19)}.

Most of the studies conducted for prevention of adhesions are related with abdominal surgery, and studies conducted for thoracic surgery are rare. Pleural adhesions following thoracotomy are beneficial when there are pleural effusions and the possibility of air leak, with a pleurodesis effect. However, in later stages, pleural adhesions may complicate the indications for re-thoracotomy\textsuperscript{(20)}. The degree of adhesions can be decreased with videothoracoscopic interventions (VATS) in patients who are candidates for a re-thoracotomy.

However, for lung cancers that are centrally located, thoracotomy is preferred over VATS. These patients may require a re-thoracotomy in later stages.

![Fig. 3](image1.png)

**Fig. 3**: Microscopic images of the rats (a. group 2. The adhesion site between the visceral and parietal pleura was observed. A mild fibrosis was seen in both pleurae. Myxoid degeneration is not present (HEx100). b. Group 3. The adhesion site between the visceral and parietal pleurae and myxoid degeneration is shown (HEx40). c. Moderate mesothelial hyperplasia, fibrosis and mild chronic inflammation of the visceral pleura was observed (HEx100). d. group 2. The mesothelium in the parietal pleura is normal, and significant chronic inflammation, vascularization or fibrosis were not observed (HEx40)).

![Fig. 4](image2.png)

**Fig. 4**: Microscopic images of the rats (a. group 2 adhesion area. Mesothelial hyperplasia (arrow) and fibrosis can be seen in the parietal pleura (HEx40). b. group 3. Marked mesothelial hyperplasia, chronic inflammation, vascularization and edema in the visceral pleura can be observed (HEx100). c. group 3. Immense myxoid degeneration in the parietal pleura on the right, and foreign body reaction on the left can be observed (HEx200). d. group 2. Despite the mesothelium is normal in the visceral pleura, a marked myxoid degeneration (arrow) and chronic inflammation can be observed (HEx200)).

Any surgery performed on the body results in various degrees of adhesions. The abdomen is the site where adhesions are most frequent and may require an emergency reoperation. Postoperative adhesions increase morbidity and mortality and result in severe economic problems\textsuperscript{(19)}. In contrast to the complications caused by post-laparotomy adhesions, no serious complications are expected in the
early phase of thoracic surgery. For this reason, adhesion-preventive agents are not used in routine clinical practice. Most previous studies in thoracic surgery are related to pleurodesis, and there are very few studies on the prevention of adhesions.

One reason is the fact that adhesions after thoracic surgery are a desired condition in the early postoperative phase. This condition forms a natural pleurodesis effect and stops possible air leaks and hemorrhages, and decreases the pleural effusion. On the other hand these adhesions result in severe problems when repeated thoracotomies are necessary, as in metachronous or metastatic lung cancer. In addition, patients with advanced stage emphysema who are candidates for lung transplantation may undergo lung volume reduction surgery to decrease their symptoms before transplantation. There are previous reports describing complications during lung transplantation caused by adhesions after lung volume reduction surgery, including massive bleeding and subsequent deaths, and phrenic nerve injuries\(^\text{20-22}\). These complications indicate the use of adhesion preventing agents in selected cases. Defining these selected patients and determining the indications of anti adhesion substances is significant information that we did not see in the relevant literature. We therefore planned this study.

In our opinion an appropriate adhesion preventive agent should be used in all patients who will undergo a thoracotomy at an early age and who are proposed to receive an at least two staged lung or thoracic wall surgery, or patients who have metastatic lung cancer with high risk of recurrence. It may be also used in patients with low respiratory reserves or bleeding diathesis to prevent the air leak and hemorrhage that may be caused by repeated thoracotomies.

The selection of anti adhesive agent is another important point. A good anti adhesive agent should not interfere with wound healing, not form a basis for infection or create a foreign body reaction, it should prevent fibrosis, continue its presence in the tissue during normal healing, be removed easily from the body after healing, and be available for laparoscopic/thoracoscopic techniques. Although there are numerous agents in the market for the prevention of adhesion, their use in thoracic surgery is limited. We used Contractubex® in this study because it possessed most of the above stated features and was readily available.

In this study, animals in group 2 with the adhesion model that we developed had a longer and more severe adhesion compared to those in group 1 in which only a thoracotomy was performed. This shows the efficacy of the adhesion model.

Also, 1 rat out of 4 in group 1, 5 rats out of 8 in group 2, and 2 rats out of 8 in group 3 developed adhesions. Despite the decrease in adhesions in group 3, this was not significant.

Statistical evaluation showed that the adhesion length and score were significantly lower in group 3. The difference between the adhesion scores were considered to result from a foreign body reaction.

### Conclusion

It has been determined that the use of cepae extractum-heparin-allantoin following thoracotomy reduces the pleural adhesions, but it does not provide a solid efficacy. Although the use of cepae extractum-heparin-allantoin gel did not totally eliminate the adhesions, it provided a decrease. We believe that their application, even only to the back of the thoracotomy incision that has the greatest amount of adhesions, will decrease the adhesions and thereby the operative time and morbidity.

### References


