Determination of In Vivo efficacy and safety of zeolite as a new pleurodesis agent

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ABSTRACT
Pleural effusion, the pathological condition in which an abnormal amount of pleural fluid is accumulated in the small space between the visceral and parietal pleurae of the lungs, can be treated by pleurodesis, whereby the pleural space is obliterated. This effect can be achieved by chemical pleurodesis utilizing various reagents such as talc, an agent commonly employed in pleurodesis. Zeolites, microporous tectosilicates found in nature as minerals, can be used in a wide range of medical applications. Different zeolite compounds may exhibit variable efficacy and safety profiles, mainly depending on their particle size. In this study, we evaluated the efficacy and safety of zeolite pleurodesis. New Zealand rabbits were administered 400 mg/kg of either agent dissolved in 2 mL of isotonic saline solution by injection into their pleural cavity, and computed tomography images were obtained on postoperative day 26. Euthanization was conducted at the end of 28 days for histopathological evaluation. Furthermore, subacute toxicity and mutagenicity profiles of zeolite were analyzed. Our findings revealed that zeolite was able to induce an adequate inflammatory response to achieve successful pleurodesis. The adhesion profiles were in favor of zeolite when compared to talc pleurodesis. Moreover, none of the tested doses of zeolite induced subacute toxicity or mutagenesis. Collectively, our results suggested zeolite as an effective and safe pleurodesis agent.

1. Introduction
In humans, the average volume of pleural fluid that should be present between the parietal and visceral pleurae is around 8.4 ± 4.3 mL. Maintenance of this amount depends on the normal activities of the fluid sources such as capillaries in the pleura, interstitium of the lung, intrathoracic lymphatic system, intrathoracic blood vessels, or peritoneal cavity [26] and the balance between the production and reabsorption rates [39]. Disruption of this balance leads to the accumulation of an abnormal amount of pleural fluid within the pleural space, causing the pathological condition defined as pleural effusion [15]. The causes of a pleural effusion may be either transudative, such as cirrhosis and nephrotic syndrome, or exudative, such as infection, inflammation, or malignancy [15,26,39].

Pleural effusions tend to be asymptomatic at the time of diagnosis, despite the occasional symptoms of dyspnea, dry cough, and pleuritic chest pain. Until now, pleural effusions have been treated via surgery, drainage by using intercostal tubes or intrapleural fine catheters, or pleurodesis [15]. In pleurodesis, the pleural space is obliterated by sticking the parietal and visceral pleura together with an attempt to prevent the relapse of pleural effusion and pneumothorax [35]. The process depends highly on the inflammatory response, leading to a decrease in fibrinolytic activity, stimulation of the mesothelial damage, and fibroblast proliferation in the pleura. Elimination of the pleural space helps decrease the symptoms and improve the patient’s quality of life. Using either chemical or mechanical methods, pleurodesis can be performed for patients with both malignant [25,35,6] and non-malignant pleural effusions [10,34]. The most commonly used chemical pleurodesis agents include povidone-iodide [11,30], bleomycin [28], silver nitrate [32,36], tetracycline [1,32], autologous blood

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Table 1
Experimental design.

| Group          | Day 0                          | Day 26                        | Day 28                        |
|---------------|--------------------------------|-------------------------------|-------------------------------|
| Zeolite (N = 5) | Administration of 400 mg/kg zeolite solution | CT                            | Euthanization                 |
| Talc (N = 5)   | Administration of 400 mg/kg talc solution | CT                            | Euthanization                 |
| Control (N = 5) | Administration of vehicle       | CT                            | Euthanization                 |

CT, computed tomography

Fig. 1. Changes in the body weights of Sprague–Dawley rats in the control and zeolite groups during the 28 days following administration.

Table 2
Hematological parameters of Sprague–Dawley rats in the control and zeolite groups. Data are presented as mean ± SEM.

| Parameter   | Reference value | Control group (N = 5) | Zeolite group (N = 20) |
|-------------|-----------------|-----------------------|------------------------|
| WBC (10⁹/µL) | 3 – 14          | 12.40 ± 0.51          | 8.40 ± 0.60            |
| LYM (%)     | 65 – 87         | 72.60 ± 3.39          | 78.90 ± 1.05           |
| RBC (10⁹/µL) | 5 – 9.5         | 7.78 ± 0.91           | 7.765 ± 0.24           |
| HGB (g/dL)  | 10 – 16         | 14.60 ± 0.93          | 14.80 ± 0.50           |
| HCT (%)     | 39 – 52         | 51.60 ± 2.73          | 54.20 ± 1.33           |
| MCV (fL)    | 48 – 56         | 51.60 ± 1.08          | 51.85 ± 1.01           |
| MCH (pg)    | 11 – 19         | 15.20 ± 1.93          | 15.60 ± 0.55           |
| MCHC (g/dL) | 25 – 35         | 31.20 ± 4.82          | 31.85 ± 0.88           |
| PLT (10⁹/µL)| 480 – 1992      | 591.20 ± 69.59        | 775.60 ± 24.13*        |
| MPV (fL)    | 5.2 – 13.1      | 11.34 ± 0.63          | 10.30 ± 0.69           |
| PDW (%)     | 5.7 – 23.9      | 16.20 ± 1.12          | 16.04 ± 1.10           |

HCT, hematocrit; HGB, hemoglobin; LYM, lymphocyte; MCH, mean corpuscular hemoglobin; MCHC, mean cell hemoglobin concentration; MCV, mean cell volume; MPV, mean platelet volume; PDW, platelet distribution width; PLT, platelets; RBC, red blood cell; WBC, white blood cell. Bold text indicates values above the reference range. *p < 0.05 vs. the control group

Although talc is the most commonly used pleurodesis agent, the side effects induced by this agent limit its clinical applications [14,17,31,38]. Thus, there is a pressing need for the development of novel pleurodesis applications with good efficiency and improved safety. In this study, we aimed to evaluate the efficacy of zeolite pleurodesis in comparison to talc pleurodesis in vivo, using New Zealand rabbits as the model organism. Moreover, the toxicity profile of zeolite was investigated by mutagenicity and subacute toxicity analyses.

2. Materials and methods

2.1. Safety profile of zeolite

2.1.1. Subacute toxicity test

Subacute toxicity was evaluated according to the International Organization for Standardization (ISO) guidelines which define adverse events occurring from 24 h to 28 days upon single or multiple administrations of a test dose of an agent. The experimental group size is 10 animals (5 for each gender) per group, and the analysis involves changes in animal weight, clinical observations, pathology, and histopathology. A total of 25 Sprague–Dawley rats were anesthetized by intraperitoneal injection of 80 mg/kg ketamine (Ketasol (10 %), Richter Pharma ag) and 5 mg/kg xyline (Rompun (2 %), Bayer). Then, the right hind legs of the animals were completely shaved and disinfected. Regarding the guidelines, 20 Sprague–Dawley rats (10 males and 10 females) received 0.5 g zeolite (Sigma, 96096) in 0.5 mL formulation prepared in physiological saline solution at 1000 mg/mL concentration by administration into the gluteal muscle. Meanwhile, the control group consisting of 5 animals (3 males and 2 females) was injected with only an equal amount of the vehicle. Skin incision lines were sutured with patch [7], and talc [14,37,41]. However, nearly 40% of patients with malignant pleural effusion experience recurrence of the disease, which poses a challenge yet to be overcome [3,12].

Zeolite microporous tectosilicates are found in nature as minerals; however, they can also be synthesized under laboratory conditions [5]. Presently, there are more than 40 naturally occurring and 229 uniquely synthesized zeolite derivatives. Zeolites mainly constitute aluminum or silicon tetrahedra blocks bound together in different conformations by an oxygen atom [2]. Distinct forms of zeolite have been used in a variety of medical and biotechnological applications [5]. Our group obtained the first patent for the use of a zeolite in pleural effusion or pneumothorax in 2018 (Publication No: US/20178031840 A1) [4].
absorbable 4.0 silk suture. Pain management was achieved by subcutaneous administration of carprofen at a dose of 2 mg/kg for five postoperative days. At the end of 28 days, all animals were euthanized with high-purity CO\textsubscript{2}.

2.2. Mutagenicity test

Mutagenicity test strains of \textit{Salmonella typhimurium}, TA98, TA100, and TA102 (Moltox, North Carolina, USA), were used to carry out the mutagenicity assays [21]. Of these strains, TA100 and TA98 detect base pair and frameshift mutagens, respectively, while TA102 detects oxidizing mutagenic effect. Since zeolite is not metabolized in the human body, mutagenicity assay was done only in absence of metabolic activation. Zeolite sample was suspended in water and different concentrations up to 1000 µg/plate (1, 10, 100, and 1000 µg/mL) were used for mutagenicity assay. The solubility was used for determining the highest concentration of test substances. Briefly, 2.0 mL of top agar containing biotin and histidine, (Sigma, St. Louis, Missouri, USA) was poured into 13-mm sterile glass culture plates. Then, 0.05 mL of zeolite solutions (to a final concentration of 1, 10, 100, and 1000 µg/plate) were mixed with 0.10 mL of an appropriate bacterial culture in nutrient broth (HiMedia Laboratories Ltd., Mumbai, Maharashtra, India) and inoculated into minimal glucose agar plates. As a positive control, 20 µg/plate 4-nitro-o-phenylenediamine was used for TA98, 1 µg/plate sodium azide for TA100, and 0.5 µg/plate mitomycin C for TA102. Bacterial cultures were grown at 37 °C for 48 h in dark, and revertant colonies were counted following the incubation period.

2.3. Application of zeolite for pleurodesis

2.3.1. Housing of animals and experimental design

Male New Zealand rabbits were obtained from Yeditepe University Medical School Experimental Research Center (YUDETAM). All animals were housed in cages at controlled temperature (21 ± 1 °C) with a 12:12 h light-dark cycle. Animal experiments were performed in accordance with the ARRIVE guidelines, and the experimental protocol was approved by Yeditepe University Ethics Committee for Animal Research (Decision Number: 744–2).

Animals were randomly divided into three distinct groups (zeolite, talc, and control) consisting of five animals (Table 1). The test groups

![Fig. 2. Histopathology of the kidney upon administration of Sprague-Dawley rats with 0.5 g (1000 mg/mL) zeolite. Tissue samples were stained with H&E. Black arrowhead indicates kidney corpuscle.](image-url)
received 2 mL of 400 mg/kg zeolite (particle sizes of less than 20 µm) or talc (particle sizes around 15 µm; Dispofarma) dissolved in isotonic saline solution, while the control group received only an equal volume of the vehicle.

2.4. Surgical procedure and computed tomography

A mixture of 35 mg/kg ketamine (10 % Ketasol, Richter Pharma ag) and 7 mg/kg xylazine (2 % Rompun, Bayer) was injected im to anesthetize the animals. The surgical site on the right lateral thoracic wall was shaved and disinfected with batticon (Adeka, Turkey) and ethanol, and the surgical area was covered with sterile sheets. A 3–4 cm incision was made medial to the tip of the right scapula. Then, muscles were separated with a mosquito clamp and intercostal muscles were perforated at a single spot. An 8/0 feeding catheter with a locked cap was inserted into the pleural space through the perforation made at the intercostal level. The test substance (zeolite, talc, or vehicle) was injected into the pleural cavity. The feeding cannula was kinked during the connection and disconnection of the syringe to prevent pneumothorax. A 4/0 vicryl suture was placed into the muscular layers around the catheter to prevent air leakage. Purse-string suture was used to stabilize the catheter.

The feeding catheter was left in the surgical area for the following four hours to ensure that any air trapped between the two pleurae has been removed. Meanwhile, the animals were kept in movement-protected cages to avoid the inadvertent dislocation of the catheters locked in the pleura. During this process, the position of the animals was changed at every 15 min to prevent the accumulation of excess air. The catheter was withdrawn simultaneously while applying the purse-string suture. This way, the skin was closed using 4/0 silk stitches.

At the postoperative stage, Amoxicillin (Clamoxyl® LA) was applied subcutaneously at a dose of 7 mg/kg (as a single dose) to prevent the potential infection risk. Carprofen (Rimadyl® XL) was applied subcutaneously at a dose of 3 mg/kg (every 24 h for three days) to prevent inflammation. Computed tomography (CT) images were obtained on postoperative day 26 using a GE HiSpeed CT/e Dual CT Scanner to investigate the potential expansion of the lungs and observe pleurodesis formation.

The animals were sacrificed on postoperative day 28 under anesthesia. Exsanguination was performed to prepare the tissues for

Fig. 3. Histopathology of the liver upon administration of Sprague-Dawley rats with 0.5 g (1000 mg/mL) zeolite. Tissue samples were stained with H&E. Black arrowheads indicate the central veins.
generalized scattered adhesions; and 3, complete obliteration of the

macroscopic and histopathological evaluations

2.5. Macroscopic and histopathological evaluations

For macroscopic evaluation of the lungs and pleural cavities of the
animals, the pleural cavity was exposed carefully by a bilateral incision
through the diaphragm, and the sternum and the medial portions of the
anterior ribs were removed. Gross pleurodesis was graded according to
the following scheme: 0, normal pleura; 1, few scattered adhesions; 2,
modified scattered adhesions; and 3, complete obliteration of the
pleural space by adhesions.

Histological examinations of the tissues obtained after dissection of
the Sprague–Dawley rats and New Zealand rabbits were conducted at
Yeditepe University, Histology and Embryology Department of the
Faculty of Medicine. Paraffin-embedded tissue samples were sectioned
at a 5-µm thickness, and Hematoxylin and Eosin (H&E) and Masson’s
tricrome staining was carried out. All samples were investigated using

Table 4
Mutagenicity of zeolite in Salmonella typhimurium TA98, TA100, and TA102 strains.

| Number of revertant/plate | TA98 | TA100 | TA102 |
|--------------------------|------|-------|-------|
| Negative control         | 28.9±2.8 | 171.0 | 392.3±48.3 |
| Positive control         | 765.0±60.7* | 913.7±64.8* | 1080.7±116.8* |
| Dose (µg/plate) 1000     | 32.2±6.1 | 172.5±4.9 | 391.0±29.7 |
| 100                      | 30.2±8.4 | 164.7±15.5 | 395.5±19.1 |
| 10                       | 26.2±6.6 | 172.0±17.0 | 384.0±33.9 |
| 1                        | 24.8±6.9 | 182.5±12.0 | 405.5±30.4 |

Table 5
Microscopic and macroscopic evaluations of the pleura.

|                            | Macroscopic Score (mean ± SEM) | Microscopic Score (mean ± SEM) |
|---------------------------|---------------------------------|-------------------------------|
| Control group (N = 5)     | 0 ± 0                           | 2.2 ± 0.73                   |
| Talc-administered group (N = 5) | 2 ± 0.316**                   | 9.4 ± 0.87***               |
| Zeolite-administered group (N = 5) | 2.6 ± 0.244***               | 6 ± 0.31**                  |

a * < 0.001 and + + + P < 0.001 vs. control group; **P < 0.01 vs. talc-
administered group

Fig. 4. Thoracic coronal (a) and sagittal (b) CT sections of the subject in parenchymal window showing full expansion of both lungs. Thoracic axial CT sections of the subject in parenchymal window at the apex (c), subcarinal (d), and diaphragmatic levels (e), all of which show full expansion of both lungs.
levels (31.10 ± 1.72 g/L; reference range: 15 – 28 g/L).

None of the animals showed abnormal clinical signs; respiration, motor skills, reflexes, muscle tone, and gastrointestinal activity of the animals were normal. In addition, convulsions, ocular symptoms, cardiovascular symptoms, salivation, piloerection, and analgesia were not observed in any of the groups. The skin, body cavities, cranial cavity, thoracic cavity, and abdominal cavity appeared normal, and no necropsy was observed.

Connecting tissues in the kidneys of the zeolite group animals were observed to be normal (Fig. 2). No pathological alterations were observed in the proximal and distal tubular epithelium forming the kidney parenchyma. The histology of glomerulus and renal capsule forming the renal corpuscle were normal. Angiogenesis was observed in the connective tissue found in the interstitial region. Histological alterations observed in the zeolite group were similar to those in the control group.

The connective tissue structure of the liver capsule was observed to be conserved (Fig. 3). The histology of hepatocytes and sinusoid capillary found in parenchyma were normal. Blood accumulation was found in the central veins of the lobes, but not throughout the whole tissue. Arterial and venous structure, as well as the structure of the bile duct, appeared normal.

3.2. Mutagenicity of zeolite

High frequencies of revertant colonies were observed in the positive control groups, compared to the negative controls for TA98, TA100, and TA102 strains, respectively. (p < 0.01) (Table 4). None of the tested concentrations of zeolite induced a significant increase in the number of revertant colonies of TA98, TA100, and TA102 strains, compared to those in the negative control groups. In addition, zeolite did not affect bacteria viability, indicating the absence of toxicity up to 1000 µg/plate.

3.3. Computed tomography

CT images were studied to ensure that no air was trapped or fluid
accumulated in the pleural space. The lungs were completely expanded, and none of the CT scans showed pleural free air or fluid (Fig. 4).

3.4. Macroscopic and microscopic evaluations of zeolite pleurodesis

Gross necropsy examination (Table 5) revealed significant obliteration of the pleural space for both the zeolite (complete obliteration, average score 2.6) and talc groups (generalized scattered adhesions, average score 2), compared with the control group (normal pleura, score 0).

H&E staining of the zeolite group right lung revealed edema, bleeding, and slight thickening in the fibrous tissue of the pleura (Fig. 5). Thickening was observed in the alveolar wall. Masson's trichrome staining showed thickening in the fibrous tissue between the parietal and visceral pleura, as well as pleural edema (Fig. 6). Bleeding was also observed in alveolar veins.

H&E staining of the talc group right lung revealed infiltrations and thickening in the visceral pleura due to the increase in the fibrous tissue (Fig. 7). Alveoli of the lung were swollen, and bleeding was observed. Masson's trichrome staining showed thickening in the parietal pleura due to the increase in the fibrous tissue (Fig. 8). Swollen alveoli and bleeding were evident.

H&E (Fig. 9) and Masson's trichrome staining (Fig. 10) of the control group right lung showed normal alveolar structure and bronchial walls.

Histopathological examination of the right pleura revealed significantly higher scores for both zeolite- and talc-administered groups, compared with the control group (Table 5). The right pleura of the zeolite group exhibited less edema, bleeding, inflammation, and fibrosis than those of the talc group. Detailed scoring of the parameters for the right pleura can be found in Table 6. There was no significant difference in the left pleural pathological scores between the talc and zeolite groups (5.80 ± 0.58 vs. 5.40 ± 0.40; Supplemental Table 1).

Regarding the examination of the right lungs that were subjected to intrapleural injection, the talc group showed a significantly lower pathological score than the zeolite group (8.00 ± 1.00 vs. 11.00 ± 1.73). Detailed scoring of the parameters for the right lung can be found in Table 7. Furthermore, the left lung tissues of the animals were subjected to histopathological examination. Although each agent was administered through the right lungs of the animals, histopathological scores of the left lung tissues were similar to those of the right lungs.
Many chemical and biological agents have been tested for pleurodesis [29]. Talc is frequently used as a pleurodesing agent due to its high efficacy, cost-effectiveness, and widespread accessibility. According to the results of an earlier study, talc slurry was an effective pleural sclerosant in rabbits in comparison to tetracycline derivatives [20]. The findings state that the degree of microscopic fibrosis increased in parallel with the incremental doses of talc, and the scores were similar to that of tetracycline derivatives. As a result, talc slurry was reported as an effective pleural sclerosant in rabbits, which did not produce hemothorax as opposed to the effects of tetracycline derivatives.

In a comparative study, safety and efficacy of three sclerosants, talc, doxycycline, and autologous blood, were investigated. All three agents produced remarkable histologic alterations at the site of pleural surface. However, autologous blood displayed no remarkable pleurodesis in short term. The levels of liver transaminases were elevated upon doxycycline pleurodesis. Moreover, remarkable angiotensin converting enzyme activity was observed upon talc pleurodesis. Compared to other agents, talc demonstrated fewer acute side effects. Thus, talc was considered a safer agent in clinical practice [23].

Another study reported the comparison of erythromycin, talc, doxycycline, and diazepam sclerosing activities [22]. Here, talc slurry was found to be ineffective in inducing pleurodesis. The authors attributed this result to two possible reasons: chest tube insertion was not performed, which could have facilitated apposition between the parietal and the visceral pleura, and the dosage of talc (70 mg/kg) was slightly below the median effective dose reported in the previous animal studies (50–400 mg/kg).

Despite the relative efficiency and safety of talc as a pleurodesing agent, it has several drawbacks. One of the remarkable side effects has been reported as adult respiratory distress syndrome in 3–9% of cases after intrapleural administration [29]. Other side effects were reported as fever, pain, arrhythmia, sclerosis, arterial desaturation syndrome [16]. Moreover, carcinogenesis has also been reported in mice [27].

Zeolites have been reported as suitable materials for medical applications, including neoplastic agent encapsulation [14,42] and anti-neurodegenerative activities [24]. Various zeolite-based materials have been reported to have a good blood coagulation activity [18,19].

Fig. 7. Representative H&E staining images of the talc group right lung. White arrow indicates the bleeding regions. Black arrow indicates infiltrations. Black asterisk indicates edema. Scale bars in panels represent 200 µm (a), 100 µm (b), 50 µm (c), and 50 µm (d).
Moreover, zeolites have been reported as an adsorbent for xenobiotics [33]. The idea that zeolite may act as a safe sclerosing agent was suggested by our research group, and the first patent for its use in pleural effusion or pneumothorax was obtained in 2018 (Publication No: US/20178031840 A1) [4].

In order to decide whether zeolite is a safe agent to be used in pleurodesis, mutagenicity and subacute toxicity tests were performed. In the mutagenicity test, the number of the revertant colonies were similar in the zeolite and control groups, which indicated that zeolite up to 1000 µg/plate did not cause mutagenicity. In the subacute toxicity test, nearly all hematological parameters were in the normal range, except hematocrit, which may be due to the slight increase in the levels of liver enzymes [13]. Collectively, these results suggested zeolite as a safe pleurodesing agent.

In this study, we investigated the potency of zeolite as a pleurodesing agent. Rabbits were selected as the suitable animal model in this research due to several reasons. First, despite their easy handling and cost-efficiency, the incomplete mediastina of mice allow the two pleural cavities to communicate freely, which prohibits the use of the contralateral pleura as a control. Additionally, intrapleural injection is more difficult, and the amount of biological material that can be obtained for examination is limited due to the small size of these animals. Furthermore, although rabbits have a thin visceral membrane, they are the most commonly employed animal models in studies regarding pleurodesis, which facilitates the interpretation of experimental results. Nevertheless, it should be noted that the thin visceral membrane of rabbits resembles humans to a lesser extent compared to larger animals such as sheep. This difference between the anatomies of rabbits and humans potentially has an influence on pleural transport processes [40].

Given the wide application of talc pleurodesis, we compared the efficacy of zeolite pleurodesis with that of talc. Regarding the previous studies that have reported adult respiratory distress syndrome in patients who underwent talc pleurodesis with a particle size of less than 10 µm [8], we preferred to use talc with a particle size of 15 µm. The larger size of talc particles prevents extrapleural dissemination to some extent, i.e., the pulmonary and systemic spread, reducing the risk of severe adhesion between the diaphragm and the liver. Indeed, the lungs of rabbits in the talc pleurodesis group exhibited significantly less edema and bleeding compared with those of the zeolite group. We also attribute the edema and bleeding observed upon zeolite pleurodesis to the

![Representative Masson’s trichrome staining images of the talc group right lung. Black arrowhead indicates the parietal pleura. White arrowhead indicates the visceral pleura. White asterisk indicates fibrous tissue. White arrow indicates the bleeding regions. Scale bars in panels represent 200 µm (a), 100 µm (b), 50 µm (c), and 50 µm (d).]
heterogenous particle sizes of zeolite used in this experiment (<20 µm). As observed in a previous talc pleurodesis study [9], small particles have a tendency to become widely distributed throughout both lungs independent of the site of injection, alongside the spleen, liver, and kidney. This extrapleural dissemination might well be one of the contributing factors to the observed pathologies in the present study. A more homogenous mixture of zeolite with larger particle sizes, similar to the 15-µm talc used in this study, should be tested to confirm this possibility. Importantly, our implications remain limited in that the deposition of zeolite particles was not analyzed; this point should also be considered in future studies. An interesting finding of the study was that the left pleural pathological score did not differ between the talc and zeolite groups, possibly due to differences in the distribution mechanisms involved. Therefore, distribution studies will also be required to understand the mechanistic impact of such differences regarding the clinical efficacy of zeolite pleurodesis. It should be noted that the observed lung pathologies in the present study may be due to the thin visceral membrane of rabbits, and the responses observed for certain particle sizes may slightly vary for humans.

Histopathological examination of the pleura showed a significantly higher increase in connective tissue filaments, bleeding, edema, and pleural thickness in the talc group, compared to those in the zeolite group. These results imply that, in the pleura, talc induced stronger fibrosis and inflammation than zeolite. A sufficiently strong inflammatory reaction is required to achieve pleurodesis [8]; otherwise, the inflammation may heal with complete restoration of the normal pleura, causing a failed pleurodesis [40]. However, in our case, the stronger inflammation triggered by talc did not mean a more complete pleurodesis. Inflammation and fibrosis were also evident in the pleural pathology of the zeolite group, indicating that the extent of the inflammatory response activation triggered by administration of zeolite was adequate for a successful pleurodesis.

According to the macroscopic evaluations, there was a significant advantage of zeolite pleurodesis over talc pleurodesis in terms of the adhesion profiles. These findings pointed out that zeolite was indeed a more potent pleurodesing agent than talc, as evident by a nearly complete obliteration of the pleural space. The normal pathology of the vehicle control group indicated that the inflammatory changes observed in the zeolite- and talc-administered pleura were indeed caused by these agents. CT images were obtained for each animal to ensure full expansion of lungs postoperatively, as well as to detect the presence of any significant parenchymal infiltration or atelectasis, which proved the absence of both conditions.

5. Conclusion

Our findings showing that zeolite can trigger complete obliteration of pleural effusions while the same dose of talc can only result in generalized scattered adhesions imply that zeolite may be a more effective pleurodesis agent. Although zeolite pleurodesis caused a
relatively worse pathology in the lungs, the pathology of the pleura was better compared with talc pleurodesis. Based on the evidence that the lesser inflammatory response in the pleura caused by zeolite was sufficient to achieve successful pleurodesis, we deduced that lower doses of this agent might also induce complete pleurodesis while reducing toxicity in the lungs. Additionally, using larger zeolite particles or other physicochemical derivatives of zeolite might help to improve the efficacy of pleurodesis, with reduced extrapleural dissemination and thus an improved safety profile compared with talc.

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Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability
Supplementary data file has been uploaded in this submission platform.

Table 6
Scoring of the histopathological parameters for the right pleural tissues.

|                      | Zeolite (N = 5) | Talc (N = 5) | Control (N = 5) |
|----------------------|----------------|--------------|-----------------|
| Fibrosis             | 1.6 ± 0.55     | 2.8 ± 0.45   | 0.4 ± 0.55      |
| Edema                | 1.4 ± 0.55     | 2.2 ± 0.84   | 0.4 ± 0.55      |
| Bleeding             | 1.4 ± 0.55     | 2.8 ± 0.45   | 1.0 ± 0.71      |
| Mononuclear cell infiltration | 1.6 ± 0.55 | 1.6 ± 0.89   | 0.4 ± 0.55      |
| Total score          | 6.0 ± 0.71     | 9.4 ± 1.95   | 2.2 ± 1.64      |

0, absent; 1, mild; 2, moderate; 3, severe

Table 7
Scoring of the histopathological parameters for the right lung tissues.

|                      | Zeolite (N = 5) | Talc (N = 5) | Control (N = 5) |
|----------------------|----------------|--------------|-----------------|
| Atelectasis          | 1.8 ± 0.45     | 1.0          | 0.2 ± 0.45      |
| Fibrin deposition in the airways | 2.2 ± 0.45 | 0.8          | 0.6 ± 0.55      |
| Bleeding in the airways | 2.0 ± 0.71 | 2.4          | 1.4 ± 0.55      |
| Alveolar capillary congestion | 2.0 ± 0.00 | 1.4          | 1.4 ± 0.55      |
| Mononuclear cell infiltration and alveolar wall thickening | 1.6 ± 0.55 | 1.2          | 0.4 ± 0.55      |
| Leukocyte infiltration | 1.4 ± 0.55    | 1.2          | 0.4 ± 0.55      |
| Total score          | 11.00 ± 1.73   | 8.0          | 4.4 ± 1.14      |

0, absent; 1, mild; 2, moderate; 3, severe

Fig. 10. Representative Masson’s trichrome staining images of the control group right lung. Black arrowhead indicates the visceral pleura. Alveolar structure and bronchial walls have a normal histology. Scale bars in panels represent 200 µm (a), 100 µm (b), 50 µm (c), and 50 µm (d).
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AA, SE, and NB designed the study. AA is the guarantor of the content of the manuscript. ES carried out the subacute toxicity and animal studies and prepared the initial draft. MH performed the mutagenicity tests. AC performed the histopathological analyses. All authors critically revised and agreed to the final version of the manuscript. The authors would like to thank all employees of Yeditepe University Faculty of Medicine Experimental Research Center for their support in housing and handling of the animals.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxrep.2022.09.003.

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