Mucolitic, Alkaline and Antiviral Properties of Potassium Hydroxide

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Abstract

Introduction: Although ash has been used for treatment and cleaning among the people since Avicenna, its use for therapeutic purposes is not common in modern medicine. The main ingredient of ash is potassium hydroxide (KOH).

Methods: In this paper, the antiviral properties of KOH were studied in vivo and in vitro conditions in mucolytic, alkaline and enveloped viruses that cause respiratory tract disease. For this purpose, a 6-stage study was planned. The physicochemical properties of the highest dose of KOH, whose caustic properties are well known, that can be used orally in humans, and the changes in the structure of mucus were investigated. Then, interactions of KOH with the membrane phospholipid bilayer complex, mucin 5AC, corona viruses spike glycoprotein, TMPRSS2 and human ACE2 (hACE2) receptors, and neuraminidase active site in influenza virus were investigated in silico, and the toxicity and beneficial properties of KOH in cells, both in vitro and in vivo, were evaluated.

Results: It has been shown that at the applied doses, KOH has a mucolytic effect and increases the pH of the environment in mucus. It has been shown to prolong life span in cell culture and have no toxicity, and in the in silico study it binds to the hydrophilic part of the cell membrane, corona virus spike glycoprotein, TMPRSS2 hACE2 receptor and neuraminidase active site in influenza virus. Oral use of KOH in the form of a spray in mice had no toxic effects on the mucosa and the inhaler application has a mucolytic effect by decreasing the viscosity of mucus in the respiratory tract.

Conclusion: In light of these findings, KOH can be mucolytic, alkaline, and antiviral for enveloped viruses in the respiratory tract.

Introduction

Since Avicenna, lye has been used in the treatment of various diseases (1). The ash content varies depending on the tree or plants it is made of, but it is alkaline and contains a large amount of potassium hydroxide (KOH) (2, 3). However, ash was more commonly used in laundry and dish cleaning in ancient times. With our current knowledge, perhaps the underlying reason for the use of ash since ancient times is its surfactant and saponification properties via KOH. For this reason, inspired by this natural phenomenon from nature, it has been matched with KOH with enveloped viruses that cause disease in the respiratory tract which is the active ingredient in ash water. However, due to the possible serious harmful effects of KOH related to its caustic property (4, 5), it has not been used by the enteral route in current medical treatment practices (6, 7).

KOH is also used in the soap-making process. Soap is obtained by the reaction of vegetable and animal oils or fatty acids with strong alkali hydroxides (NaOH, KOH, etc.) (8, 9). Lipids play a central role in viral infection as they form the structural basis of cellular and viral membranes (10–12). Soap dissolves the lipid membranes of viruses and thus the virus becomes ineffective (13, 14). In meta-analyzes, it has been reported that the transmission of respiratory viruses can be prevented by 45–55% by hand washing (15,
In COVID-19 and influenza infection, the importance of hand washing with soap has been emphasized to reduce the transmission of the virus (13, 14, 17, 18).

It has been shown that coronavirus and influenza virus fusion does not occur at neutral pH and that fusion activation is a low pH-dependent process occurring in acidic endosomes. Studies have shown that while pH decreases from 7 to 5, virus-infected cells increase (19–21).

COVID-19 and influenza virus infection can lead to severe conditions that can progress to death, especially in older adults and underlying co-morbidity diseases (22, 23). Therefore, the importance of reducing the viscosity of mucus and ensuring its fluidity in survival is emphasized in COVID-19 and influenza virus infection without obstruction in the respiratory tract.

In these preclinical studies, mucolytic, alkalinization, and saponification effects of ash, KOH, and saline were evaluated in-vitro and in-vivo environments. KOH has not been used orally or nasally due to its caustic nature, which is due to the known alkalinity of KOH.

The main aim of this paper is to explain the KOH molecule effects in these pH ranges in solution for its possible usefulness in the treatment of enveloped viruses that infect the respiratory tract.

**Material And Methods**

Plan of 6 different in-vitro and in-vivo preclinical studies:

**A. In-Vitro studies:**

In the 1st study; surface tension, contact angle, and pH values of saline, KOH, and lye were studied. This study was conducted to observe the basic physicochemical properties of KOH.

In the 2nd study; saliva was used as the source of mucus and saline, KOH, and lye were added to the mucus. Changes in surface tension, contact angle, and pH values of mucus were examined. This study was carried out to observe the changes in the mucus structure of KOH.

In the 3rd study; the effects of different concentrations of KOH and lye on mitochondrial dysfunction and cell survival in cell culture were evaluated. This study was conducted to observe the potential toxicological effects of KOH.

In the 4th study, interactions of KOH with membrane phospholipid bilayer, mucin, virus components/mediators were studied in a silico simulation. This study was carried out with the aim of observing the reactions and reaction properties of the molecular agent, which is thought to have possible positive antiviral effect against coronavirus and influenza in the treatment of these diseases with simulation with artificial intelligent.

**B. In-vivo studies:**
In the 1st study in order to evaluate the possible harmful effects of KOH on injured cells, the histopathological changes caused by KOH in the damaged tissue of the oral mucosa were evaluated. This study was carried out to observe the possible damage to the oral mucosa in the case of the use of KOH.

In the 2nd study, initially, histopathological examination of the main respiratory tract was performed and mucus viscosity in mice was then examined. This study was carried out to observe the cellular damage in the lung tissue, the effect on the lung mucus, the risk of hyperkalemia in serum potassium levels in the case of the use of KOH.

1. Ethics Committee Approvals: Before the experiments, ethical approval for the animal studies was obtained from the Adana Veterinary Research Institute Ethics Committee Unit (20.04.2020-2020-1/800). Furthermore, ethics committee approval for mucus study was obtained from the non-invasive clinical research ethics committee (04.09.2020-103) of Çukurova University Faculty of Medicine.

2. Preparation of lye: The branches of fig and olive trees were collected and dried for 5 days without wetting. The twigs were then burned. After the ash has cooled, 10 g of ash is placed in 500 mL of saline in a sterile container and kept for 12 hours. After ash decantation, the supernatant was sampled and buffered in saline to pH 8.9.

3. Preparation of KOH solution: KOH solution was formed by adding KOH (Merck, Darmstadt, Germany) to 1 L distilled water containing 0.9% NaCl at pH 8.9. Solution pH was prepared to 8.90 by vortexing (VELP) at 500 rpm for 10 minutes at room temperature. The KOH molarity in the solution whose pH was fixed at 8.90 was calculated as $8.43 \times 10^{-5}$.

4. Mucus collection: Volunteers (35-50 age, male) were asked to transfer 25 mL of saliva accumulated in their mouths in the early morning hours into the container given. We studied with a single-gender in order not to affect the reactions that occur in the volunteer mucus from gender differences. The collected saliva was centrifuged at 5,000 rpm for 5 minutes (Sigma2-16K). The supernatant was divided into 4 collecting vessels: 1st tube: 7 mL, 2nd tube: 3.5 mL, 3rd tube: 3.5 mL, and 4th tube 3.5 mL. While no addition was made to the 1st tube; saline, KOH, and lye solutions were added to the 2nd, 3rd and 4th tubes, respectively. Subsequently, examinations were carried out at room temperature promptly.

5. Atomic absorption spectrometry measurement: The concentration of K and sodium (Na) elements in the liquid solution was determined by atomic absorption spectroscopy of saline, KOH, and lye solutions. Results were set in ppm (PerkinElmer precisely AAnalyst 700).

6. Study of physicochemical properties of fluids:

6. 1. pH measurement: The probe was dipped into the prepared liquid and the measurement continued until the pH meter screen was fixed at room temperature. The fixed value was accepted as the measurement of pH (Martini Mi151).
6. 2. Surface tension measurement: Measurements were made according to the Du Nouy ring method (24). The prepared 7 mL liquid samples were put into the liquid container. After the ring was immersed in the liquid, it was slowly pulled up from the liquid. After the ring was separated from the liquid, the value displayed on the screen was recorded with the measurement value Dyn/cm (TD1C LAUDA).

6. 3. Contact angle measurement: Contact angles of liquids were evaluated using the Sessile drop method. The liquid whose contact angle was to be determined is imaged with a micropipette in the form of a micropipette with a high-resolution camera (OneAttension program was used) (25). Then it was left on the microplate cleaned slide with the trigger apparatus. Then, the angles made with the surface on both sides of the liquid were recorded as contact angles. The calculation was made by taking the average of both angles (24).

7. 1. Retrieval and preparation of the receptor structures for the in a silico study: In this study, molecular docking experiments were carried out to reveal the affinity of the KOH molecule to the membrane bilayer complex, mucin5AC, 2019-nCoV spike glycoprotein, TMPRSS2, human angiotensin-converting enzyme 2 (hACE2) receptors and influenza virus neuraminidase enzyme(26-28). The KOH ligand (ID:14113) used in this study was downloaded from www.chemspider.com in the ‘mol’ format. The molecule to which hydrogen atom was added, was optimized using Avogadro software and saved in ‘pdb’ format.

7. 2. Molecular docking experiments: Molecular docking calculations were performed using AutoDock 4.2 to predict the binding affinity of KOH with the 2019-nCoV RBD, TMPRSS2, membrane bilayer complex, mucin5AC, hACE2 and influenza virus neuraminidase enzyme. AutoDockTools-1.5.6 was used to prepare the target and ligand molecules and also the parameters before initiating the docking analysis using AutoDock 4. 2(29, 30).

The best docking poses obtained using AutoDock 4.2 between the ligand and receptor structures were analyzed with the BIOVIA Discovery Studio Visualizer 2016.

8. Cell culture study: NIH-3T3 mouse embryonic fibroblast cells were used in cell culture studies. The positive or negative effects of different concentrations of KOH solution and lye (1%, 0.1%, 0.05%, 0.01%, and 0.001%) on cells were investigated. This method was made based on active absorption of 3,4,5-dimethyl-thiazolyl-2,5-diphenyltetrazolium bromide (MTT) into living cells and reduction to blue-purple colored, water-insoluble formazan (31-33).

9. In vivo studies on animals

9. 1. Oral mucosal toxicity study: 40 male 10-week-old Swiss albino mice (25-30 g) were divided into 5 groups (n = 8). Groups were formed by the random selection of mice. The experiment was modified according to the principles of oral mucosal injury. Oral mucosal damage was created in the first 4 groups. The 5th group was formed as a control group in order to make a comparison with damaged tissue. KOH solution was applied to groups 1 and 2, and saline to groups 3 and 4 were applied as a spray in the
mouth with an interval of 8 hours for 5 days, targeting the injury site. Euthanasia was performed under anesthesia on the 5th day to the 1st, 3rd, and 5th groups, and on the 21st day to the 2nd and 4th groups. In the subjects, the part with mucosal damage was excised (34, 35).

9. 2. The effectiveness of KOH on bronchoalveolar epithelial cell damage and bronchoalveolar lavage and toxicity studies on blood K level: While groups 1 and 2 consisted of 2.5 months old (22-28 g) young mice, mice in groups 3, 4, 5, and 6 consisted of 30 months old (30-35 g) advanced-aged male mice. In groups 5 and 6, the steam of KOH solution was applied with a nebulizer. No chemicals were applied to the other groups. Bronchoalveolar lavage (BAL) samples of 1st, 3rd, and 5th group mice were taken under anesthesia on the 6th day. Tissue histopathology and blood samples were obtained from mice in groups 2, 4, and 6.

9. 3. KOH application with a nebulizer to mice: Prepared KOH solution was put into the nebulizer chamber. A face mask was not attached to the end of the air tube. Each mouse was fixed in the hand, from the back and nape of the neck, allowing the nebular vapor to enter the mouth and nose (MMAD:0.5-5μm, Nebulization rate:0.25Ml/min, Compressor pressure:2.5bar Nimo; Turkey). This process was applied for 30 seconds every 8 hours for 5 days.

9. 4. BAL: The previous procedure has been modified, keeping the basic principles the same. The trachea was cannulated with a 27-gauge injector tip. The injector tip was clamped so that the distal part was inside the trachea. 0.3 mL of saline was gently injected at room temperature. Then, 0.1 mL of liquid was aspirated back (35-38). 9. 5. Serum K level: Blood was drawn from the right atrium after BAL samples were taken while the subjects were under anesthesia and serum K levels were evaluated.

9. 5. Histopathological evaluation: In histopathological evaluation, reepithelialization, granulation tissue, inflammation, and angiogenesis were evaluated in oral mucosa tissue samples. Furthermore, edema, hyaline membrane formation, neutrophil infiltration, lymphocyte infiltration, bronchial epithelial damage, and hemorrhage were evaluated for lung tissues. Scoring according to the severity of the above parameters was done as follows; 0:no pathological finding, 1:mild, 2:moderate, 3:severe (39).

10. Oxidative stress studies:

10. 1. Determination of malondialdehyde (MDA): The determination of MDA, one of the lipid peroxidation products, was performed based on the reaction of thiobarbituric acid and MDA, which is found by Ohkawa et al, to give a colored compound that can be measured at 532nm wavelength (40).

10. 2. Determination of Superoxide dismutase (SOD): In the method applied by Fitzgerald et al., superoxide radical is produced by the xanthinexanthine oxidase (XOD) system and the resulting superoxide radical reacts with lodonitrotetrazolium to form a violet-colored formazan dye and this color intensity is measured at a wavelength of 505nm (41).

10. 3. CUPRAC (CUPRIC Reducing Antioxidant Capacity): In the method developed by Apak et al., phenolic hydroxyls are transformed into quinone structures corresponding to the CUPRAC redox reaction and it is
based on the principle of measuring the Cu(I)-Nc chelate

11. Statistical evaluation: Paired Sample t-Test was used to compare dependent samples observed in two different situations. Student t-Test was used to compare the data in which independent samples were suitable for normal distribution (pH, surface tension, contact angle, serum potassium level and histopathological examination), and a non-parametric MannWhitney U test was used to compare the means of two independent groups that did not show normal distribution characteristics (mitochondrial activity analysis and oxidative stress changes). SPSS 17 package program was used in the study. p<0.05 was considered significant.

Results

Research (in-vitro) 1

In atomic absorption spectrometric analysis, the amount of K in KOH solution was determined as 2.94 ppm. The K value detected in the lye solution was 3.55 ppm. Na value was detected as 2700 ppm in all three solutions.

The prepared KOH and lye solution was more alkaline than saline, and higher surface tension and contact angle (p < 0.05) (Figure-1).

Research (in-vitro) 2: Research(in-vitro) 2

The pH value of mucus increased from 6.79 to 6.84 with saline (p > 0.05). Mucus pH was found to be more alkaline with the addition of KOH or lye solution, in which the difference was statistically significant (p < 0.001).

It was found that saline reduced the mucus surface tension(p > 0.05). KOH and lye solutions both reduced the mucus surface tension (p < 0.001). The reduction in surface tension with KOH was to a lesser extent compared to lye solution(p < 0.05). Saline, KOH and lye solutions were found to reduce the contact angle of mucus. While this difference was statistically significant in KOH solution (p < 0.05), it was not significant in saline or lye solution (p > 0.05)(Figure-1).

Research(in-vitro) 3

In the mitochondrial activity analysis, cell survival increased by 49% at 24 hours with 1% KOH solution compared to normal cells (p = 0.009). These positive findings were also found in the lye solution, but the results were not statistically significant (p > 0.05). Although lye solution was found to have a toxic effect at different concentrations, no toxic effect was detected in any concentration of KOH solution employed in this study. The statistical difference between normal cell line and pure medium was performed to determine the accuracy of our method (Figure-2).
Research (in-vitro) 4:

In the molecular docking study, binding free energy and inhibition constant values of the KOH molecule against membrane bilayer complex, mucin5AC domain, 2019-nCoV spike glycoprotein, TMPRSS2, hACE2 receptors and influenza virus neurominidase enzyme were obtained (Figure-3 and Table-1).

<table>
<thead>
<tr>
<th></th>
<th>Binding energy value (kcal/mol) (ΔG)</th>
<th>Inhibition constant mM</th>
<th>Area of interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane bilayer complex</td>
<td>-1.62</td>
<td>64.7</td>
<td>Glycerol, phospholipid, cholin, phosphate</td>
</tr>
<tr>
<td>Mucin 5AC</td>
<td>-1.74</td>
<td>52.56</td>
<td>Glu 1161, Pro 1136, Ala 1099, Thr 1103, Val 1109</td>
</tr>
<tr>
<td>Spike glycoprotein of SARS-CoV-2</td>
<td>-1.7</td>
<td>56.57</td>
<td>Glu 406</td>
</tr>
<tr>
<td>TMPRSS2</td>
<td>-1.89</td>
<td>41.33</td>
<td>Ser 460, Trp 461, Gly 462, Gly 464</td>
</tr>
<tr>
<td>Neurominidase</td>
<td>-2.05</td>
<td>31.55</td>
<td>Glu277, Arg292, Tyr347, Arg371</td>
</tr>
<tr>
<td>hACE2</td>
<td>-2.07</td>
<td>30.53</td>
<td>Asp355, Gly354, Thr 324</td>
</tr>
</tbody>
</table>

Table-1: Molecular docking study

Research (in-vivo) 1

On the 5th and 21st day of oral mucosa damage group, there was no statistical difference among the control group, saline treated group, or KOH treated group in terms of histopathological reepithelialization, granulation tissue, inflammation and angiogenesis formation (p > 0.05).

Research (in-vivo) 2

Comparison of serum K values: Both were not significantly different between the serum potassium level in the advanced aged group (Group without KOH: 5.312 mEq/L, group with KOH: 5.312 mEq/L (p > 0.05)).

Evaluation of sputum contact angle in bronchoalveolar lavage

Sputum contact angle was found to be lower in the young group compared to the advanced age group without KOH solution inhalation (p < 0.05). In the advanced age groups, sputum contact angle was found to be reduced in the KOH-inhaled group compared to the group without KOH solution inhalation (p < 0.05) (Figure-4).

Histopathological study
There was no statistical difference in histopathological edema, hemorrhage, neutrophil infiltration, lymphocyte infiltration, hyaline membrane formation and bronchial epithelial damage formation between the KOH-inhaled advanced age group and the other two groups (p > 0,05) (figure-5).

**Oxidative stress changes**

Compared to the elderly group, SOD and total antioxidant levels were higher and MDA levels were lower in the younger group and in the advanced group who received KOH(Figure-6).

**Discussion**

In this study, the results of in vitro and in vivo studies to evaluate the possible antiviral effect of KOH on enveloped viruses that can cause respiratory tract disease are discussed.

**In-vitro studies:**

In the first stage (in-vitro 1) of the study, the alkalinity of the lye and KOH solution was dependent on the alkalinity of the KOH in the lye and KOH solution. Surface tension and contact angle properties were also higher in lye and KOH solution than saline. It was interpreted that there was cohesion resulted from the interaction of the molecules in KOH and lye compared to saline.

Normal mucus activity is necessary for the continuation of a healthy life. Prevention of mucus plugs in respiratory viral infections is very important in reducing morbidity and mortality. When we look at the effects of saline, KOH, and lye on mucus obtained through saliva in the second stage (in-vitro 2) of the study.

The effect of alkalinizing, decreasing the surface tension and contact angle was detected mostly in KOH solution in 3 different solutions that interacted with mucus.

Based on the results of the in silico molecular docking study, it was thought that the interaction between glutamic acid and lysine residues of mucin 5AC polymer with KOH affects the viscosity of the mucus structure.

In this study, the decrease in surface tension and contact angle in mucus indicates that viscosity in mucus decreases. This shows us the mucolytic activity of KOH.

One of the triggering conditions for coronavirus and influenza fusion with a human cell is the acidified endosome on the cell’s surface. In other words, the virus needs a low pH environment. Rapidly progressing inflammation acidifies the environment metabolically. It is known that both metabolic and respiratory acidosis increases the entry of the virus into the cell (42). In respiratory viral infections, KOH solution will try to prevent both coronavirus and influenza entry into the cell and reduce the harmful effects of metabolic acidosis with its alkalinizing effect. The in silico study (in-vitro 4) suggests that the contacts of KOH with the active site residues of the ACE 2 receptor (Figure-3) may prevent virus fusion in
the oral area as well as in the lung tissue (43). Spike glycoprotein, which determines the diversity of coronaviruses and host tropism, emerges from the viral surface.

Structural and functional analyzes showed that the SARS-CoV-2 spike also binds to ACE2. Following the binding of SARS-CoV-2 to the host protein, the spike protein undergoes protease cleavage (44).

Also, interesting data is that Asp355 is among the subdomain I residues of hACE2 that come into contact with the SARS-CoV-2 spike glycoprotein (20). The other enveloped virus that causes disease in the respiratory tract is influenza virus. In influenza virus, the neurominidase enzyme is one of the antigenic structures of the virus and helps the antigenic component of hemagglutinin. Neurominidase has an important role in the cell entry and subsequent spread of the influenza virus. KOH has been shown to bind to the active site of the neurominidase enzyme in influenza virus, closure of this active site may block the binding of influenza virus to sialic acid. In addition to the positive effects of KOH solution on mucus, it can be thought that making the environment alkaline can prevent the virus from entering the cell differently.

Also, the attachment of KOH to the SARS-COV-2 spike glycoprotein in the receptor-binding domain (RBD) (Table-1) may be due to the electrical attraction of the amino acid residues of the RBD with KOH. This effect may also be an important step in stopping the virus fusion. The fact that spike protein forms fusion more easily in an acidic environment suggests that this fusion can be prevented by the alkaline property of KOH (45). The fact that most of the viruses that infect the respiratory tract are enveloped reveals the importance of the destruction of the envelope structure in antiviral studies.

Especially in caustic injuries that can result in death in children, it is thought that saponification in adipose tissue caused by KOH is the main physio pathological event.

In the third study (in-vitro 3), it was observed that the addition of KOH solution (1%) and lye (1%) to the cell culture reduced mitochondrial dysfunction, and this was mostly in KOH solution with a rate of 49%. It was thought that this effect may be due to the alkalinization of metabolic acidosis by KOH. Observation of toxic effects in some doses of lye (0.1%, 0.01%) may be related to the toxic effects of unidentified heavy metals and compounds in lye. Due to these possible toxicities of the lye, only the toxicity of the KOH solution was studied comparatively in the later stages of the study.

Although the exact mechanism of damage in caustic tissue injuries caused by KOH is not known, KOH rapidly penetrates the skin and saponifies the plasma membranes (46). In our in silico study, it was determined that KOH can bind membrane phospholipids by an exergonic (ΔG: -1.62 kcal/mol) reaction and this binding is on the hydrophilic side of the phospholipids (Table-1). This result suggests that when the KOH encounters the virus, saponification of the virus envelope may disintegrate the virus envelope and free the RNA or DNA strand to eliminate the viability of the virus.

In addition to the alkalinizing properties and mucolytic effects of KOH, saponification with the lipid in the virus envelope can be very effective for enveloped viruses that cause respiratory diseases. Although
tolerable pH regulation was made, in vivo study models were also included in this preclinical study to increase safety.

In the in-vivo 1st study, it was shown that topical application of KOH did not cause histopathological changes on injured oral mucosa tissues. Therefore, it was thought that the use of KOH solution locally in the oral mucosa tissue could be safe. The fact that the KOH solution is divided into very small liquid particles by spray or nebulizer may also contribute to the absence of toxic effects.

Studies designed to improve increased mucus viscosity in patients with mucoobstructive diseases identify a reduction in mucus concentration and viscosity as key therapeutic strategies (47). In the in-vivo 2nd study planned in line with this information, while no difference was observed between the histopathological findings of young mice and elderly mice, the increase in sputum contact angle in the elderly group was associated with a decrease in the hydrophilic feature of sputum and these findings were consistent with previous reports. The mucus contact angle was reduced in the advanced age group who received KOH solution inhalation compared to the group not applied. This finding was consistent with the mucolytic effect detected in the second phase of the study (48).

In the in-vivo second study, the administration of KOH inhaler did not make a histopathological difference between the two elderly groups, positively increased SOD activity and total antioxidant level, prevented MDA increase, and it was shown that KOH solution at these doses did not damage the lung tissue. The fact that the KOH application positively changes the oxidative stress findings in the elderly group shows the positive effects of inhaled KOH application on cell repair. These results show us that the KOH solution applied at pH 8.90 does not cause saponification or other tissue damage in the lung exposed to inhalation.

It can be thought that inhaled drugs will be rapidly distributed in the systemic circulation like intravenous administration. The fact that no difference was observed in serum potassium levels in the group in which KOH solution was applied suggests that this dose of KOH solution would not cause hyperpotassemia side effects.

Some viruses with envelope structure can cause disease in people of all ages in the respiratory tract. Among these, Respiratory Syncytial viruses have an important place alongside coronavirus and influenza virus. And all these viruses lose their viral effects when their envelope structure is broken.

Lipids in the membrane structure that preserves the entire vitality of the virus can form micelles with the interaction of KOH. The micelles formed will cause the membrane structure of the virus to deteriorate. This will cause the virus to lose its viability. With this potential effect, the application of KOH in viruses with enveloped structure suggests a virucidal effect.

According to the results of this study, KOH, with its mucolytic, alkalizing and potential virucidal activity, may be protective against enveloped viruses that cause respiratory tract infections.
As a result of the use of KOH in the form of a spray orally, in the nasal route, or a nebulizer, the lipid envelopes of the viruses in the oral, oropharyngeal, nasal area, and lungs can be destroyed, mucus plugs can be prevented with its mucolytic effect, environmental alkalinity can be achieved and virus fusion inhibition can be achieved. Further studies are required to use KOH as an antiviral effect on enveloped viruses.

**Ethics Committee Approvals**

Before the experiments, ethical approval for the animal studies was obtained from the Adana Veterinary Research Institute Ethics Committee Unit (20.04.2020-2020-1/800).

Furthermore, ethics committee approval for mucus study was obtained from the non-invasive clinical research ethics committee (04.09.2020-103) of Çukurova University Faculty of Medicine.

**Declarations**

**Consent to Participate:** Interventional experiments in humans have not been invasive.

**Consent to Publish:** Interventional experiments in humans have not been invasive.

**Our research does not contain any funds.**

**Declaration of Competing Interest:** The authors declare that they have no competing interests.

**References**


Figures

Figure 1

Physicochemical changes in A) liquids, B) mucus
Figure 2

The mitochondrial activity
The interaction of KOH with hACE2

The interaction of KOH with spike protein.

Intermolecular (electrostatic and hydrogen bonds) interactions of potassium hydroxide (KOH) with the neuraminidase enzyme of the influenza virus (Electrostatic interaction is given as orange and hydrogen bonds as green dashed lines. The protein (neuraminidase) was depicted in cartoon mode whereas the ligand in stick mode).

The interaction of KOH with lipid membrane

Figure 3
Molecular Docking
Figure 4

Contact angle of BAL

Histopathological images of oral mucosa damage.

Histopathological images of the lungs of the experimental groups.

Figure 5

Histopathological examination
*: Calculated according to the standard equivalent to 1 mg/ml trolox.

Figure 6

Oxidative stress alterations