RESEARCH

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Medical Science

Adverse effects of ingredients available in single nasal decongestant formulation at trachea and lungs on an experimental rat model

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Background and Aim: **There is little histologic data concerning effects of nasal decongestants on respiratory tract. We aimed to put forth the effects of mostly used ingredients of nasal decongestants on trachea and lower airways of rats.** ***Materials and Method:*** **Four-six months old 60 male rats were randomly categorized into 6 groups. Experimental drugs were applied to the same nostril twice daily for 8 weeks (Xylometazoline, Benzalkolyum, EDTA, Sorbitol and combined drug solution). Normal saline solution (NaCl %0.9) applied for the controls. At the end, trachea and both lungs were dissected and kept in formaldehyde for histopathologic evaluation.** ***Results:*** **Inflammation and bronchial edema were most common findings. All rats in sorbitol group had increased numbers of type 2 pneumocytes. Eighty percent of BAC group had increased numbers of type 2 pneumocytes. Spillover of tracheal epithelium was seen mostly in sorbitol, EDTA and combined drug groups (60%, 87.5%, 50% respectively). Bronchial smooth muscle hypertrophy was seen mostly in BAC and EDTA group (70%, 62.5% respectively). Number of goblet cells showed significant difference between control-combined drug (p=0.025) and control-BAC (p=0.001) groups.** ***Conclusion:*** **Nasal decongestants might have adverse effects such as increased airway inflammation, edema, type 2 pneumocyte numbers and epithelial desquamation mostly.**

INTRODUCTION

There is little physiologic and histologic data concerning the possible effects of nasal decongestants on respiratory tract (1). It is not known even nasal decongestants can reach to the lower airways and can make any effect. However, it is possible for this kind of drugs to reach the lower airways and the lungs at least in a small amount. Also it should be taken in to account that nasal drops can be sold as an over-the-counter (OTC) drugs and allow many patients to have self-medication. Therefore, its side effects are worth investigating for rational drug therapy approach. Nasal decongestants in the market varies widely, however the mostly used trademarks has xylometazoline as active ingredient; moreover, there are some preservative ingredients in these drugs such as benzalkonium chloride (BAC), sorbitol and ethylene diamine tetraacetic acid (EDTA). In this study, we aimed to put forth the effects of chronic administration of nasal decongestants on trachea and lower airways to see if they might have a role in exacerbations of asthma and chronic obstructive pulmonary disease (COPD).

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MATERIALS AND METHODS

Our study has been started after the permission of animal ethical committee of Adnan Menderes University (ADU-HADYEK 64583101/2015/019). All experiments were performed on 4-6 month-old male Wistar rats. Rats were housed in cages at room temperature (20±2ºC), 12 hours dark and 12 hours light within a daytime. Rats had ad libitum access to water and food which was standard pellet food. Drug application and dissection of the rats were done at Laboratory of Experimental Animals, Adnan Menderes University Medical Faculty.

In our study, 60 male rats randomized to 6 groups and weighed at the beginning. Following 8 weeks, drugs (Xylometazoline, Benzalkolyum, EDTA, Sorbitol solutions and combined form of xylometazoline and the other preservatives) were applied to both nostrils of rats with dropper twice a day, and %0.09 NaCl for the control group. Feeding status of rats had been observed. No other drugs or anesthetic agents were administered during the study.

At the end of eight weeks, rats were weighed and anesthesized with Ketamin Xsilazin (50 mg/kg + 5 mg/kg). After cervical dislocation, trachea and lungs were dissected and put into 10% formaline solution. Lungs and trachea tissues were sent to the pathology department in formaline for histopathologic evaluation.

Histological Evaluation

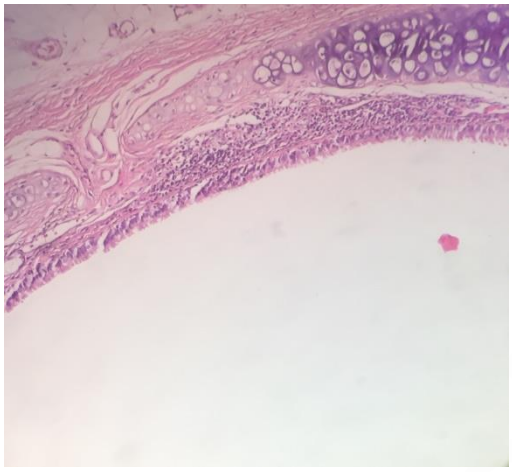
Histopathological evaluation was performed by an experienced pathologist. The trachea and lung tissues that were fixed in 10%

formalin for 24 hrs were embedded in paraffin. Tissue sections were cut at 4µm, mounted on slides, stained with hematoxylin-eosin for general lung and trachea structure, PAS-Alcian Blue Ph2.5 to identify goblet cell of trachea. All the sections were examined using a Nikon Eclipse Ci light microscope. Goblet cell hyperplasia, chronic inflammation of airway, bronchial smooth muscle hypertrophia and lymphoid follicle formation, bronchial edema, type 2 pneumocyte hyperplasia and desquamation of tracheal epithelium signs were evaluated histopathologically.

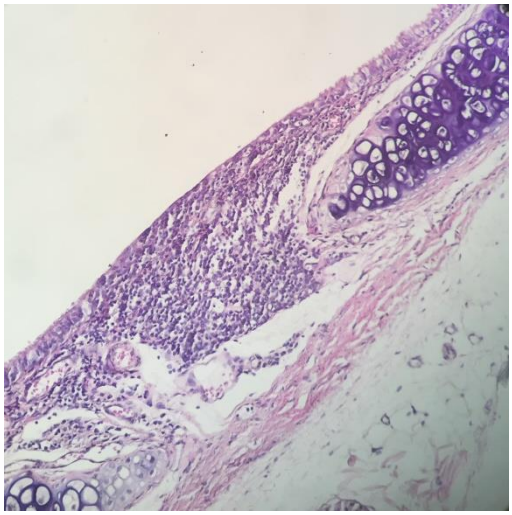
Scoring for histological findings

Chronic inflammation of trachea

Normal (Score 0 / No effect), Scattered rare lymphoid cells under the trachea epithelium (Score 1 / Mild), Lymphoid aggregates under the trachea epithelium (Score 2 / Moderate), Lymphoid cells attacking the trachea epithelium (Score 3 / Severe) (Picture 1).



Picture 1a Lymphoid cells under the surface epithelium (mild)



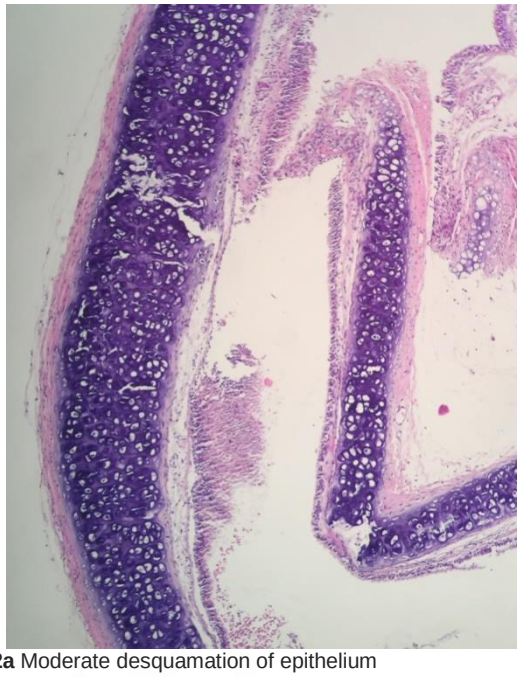
Picture 1b Lymphoid aggregates under epithelium and lymphoid cells attacking epithelium

Desquamation of tracheal epithelium

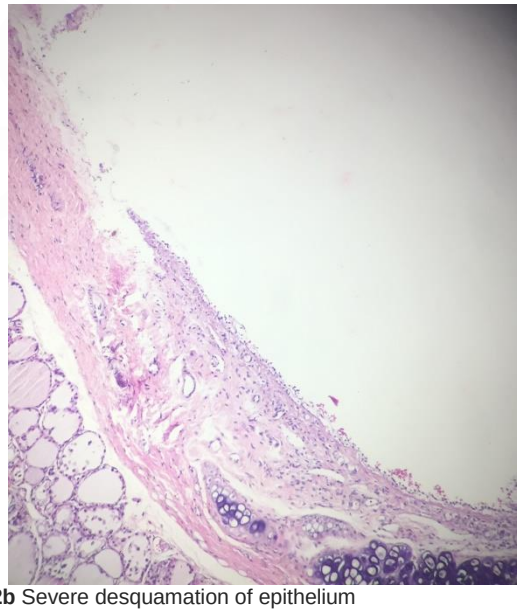
Normal (Score 0), desquamation less than 1/3 of epithelium (Score 1), desquamation 1/3 to 2/3 of epithelium (Score 2), desquamation more than 2/3 of epithelium (Score 3) (Picture 2).

Goblet cell hyperplasia

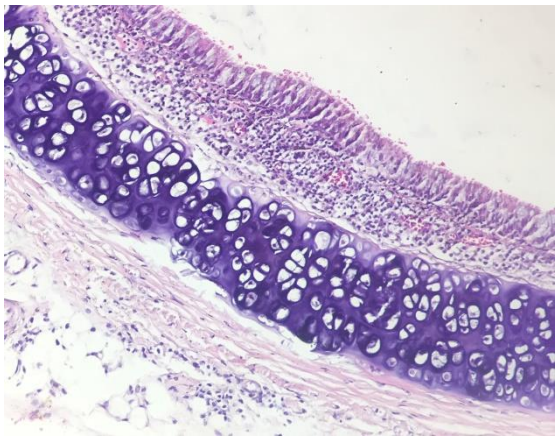
Average goblet cell count at X200 magnification field stained with PAS –Alcian Blue determined (Picture 3).



Picture 2a Moderate desquamation of epithelium



Picture 2b Severe desquamation of epithelium



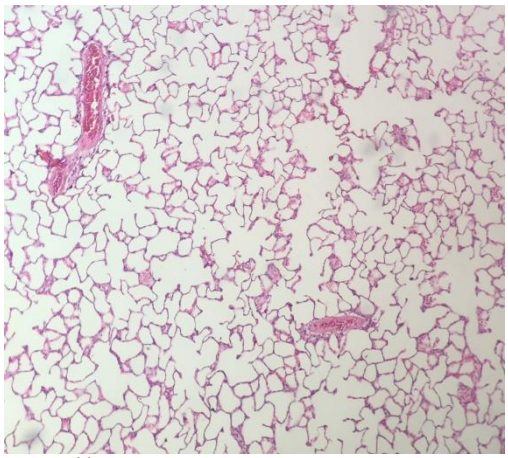
Picture 3 Severe goblet cell hyperplasia

Bronchial edema

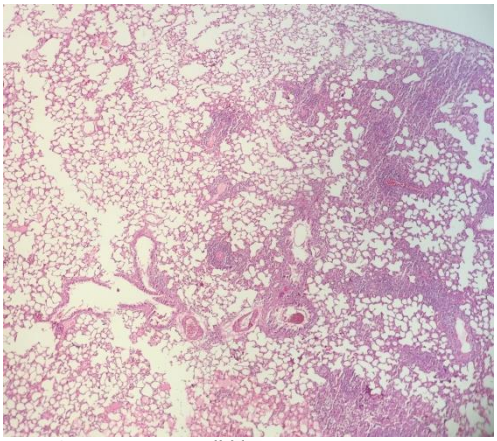
Normal (Score 0), less than 1/3 of bronchial wall affected (Score 1), 1/3 to 2/3 of bronchial wall affected (Score 2), more than 2/3 of bronchial wall affected (Score 3).

Bronchial smooth muscle hypertrophia and lymphoid follicle formation

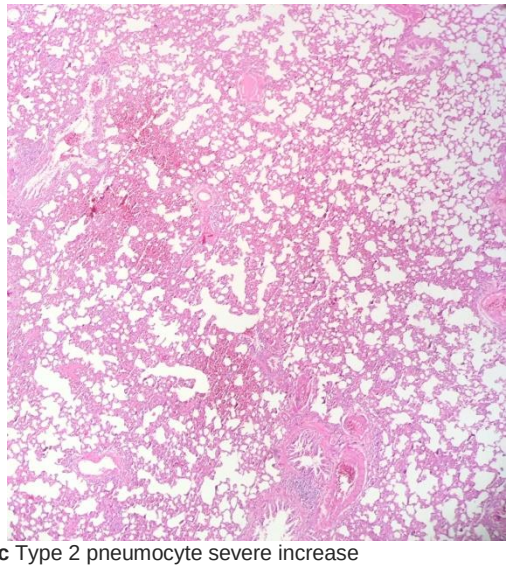
Normal (Score 0), less than 1/3 of bronchial wall affected (Score 1), 1/3 to 2/3 of broncial wall affected (Score 2), more than 2/3 of bronchial wall affected (Score 3).



Picture 4a Normal lung



Picture 4b Type 2 pneumocyte mild increase



Picture 4c Type 2 pneumocyte severe increase

Type 2 pneumocyte hyperplasia at lung paranchyme

Normal (Score 0), less than 1/3 of lung parenchyma affected (Score 1), 1/3 to 2/3 of lung paranchyma affected (Score 2), more than 2/3 of lung paranchyma affected (Score 3) (Picture 4).

Statistical analysis

Data were analyzed with SPSS (Version 22.0, SPSS Inc., Chicago, IL, USA; license, University of Hitit, Turkey). Descriptive statistics with a normal distribution are presented as mean ± standard deviation. Nominal variables are presented as number of cases and percentage (%). Kolmogorov–Smirnov and Shapiro-Wilk normality tests were used to evaluate the distributions of the groups. Levene's test used to evaluate the homogeneity of variance. The significances of the difference between more than two groups were evaluated by using Kruskal-Wallis Test since data were not normally distributed. In order to determine which groups differ, post-hoc tests were used. The Spearman rank-order correlation coefficient, kendall tau-c and Goodman-Kruskal Gamma was used to calculate correlation between two ordinal variables. P value <0.05 was considered statistically significant.

RESULTS

When all groups were evaluated, the most common findings as a result of drug application were tracheal inflammation and bronchial edema (Table 1). Chronic inflammation increased in all groups except controls. Mild, moderate and severe effects can be seen at Table 2 and Figure 1a. Bronchial edema was found mostly in the sorbitol, xylometazoline, BAC and EDTA groups at different severity levels (Table 2, Figure 1). For combined drug group, only 20% of rats had bronchial edema (Table 2, Figure 1b). All rats in the sorbitol group showed an increase in type 2 pneumocytes (60% mild, 30% moderate, 10% severe); whereas in benzalkonium group, 80% of rats had increased type 2 pneumocytes (30% mild, 40% moderate, 10% severe) (Table 2) (Figure 1c). Desquamation of tracheobronchial epithelium was observed in 60%, 87.5%, 50%, 30% and 30% of sorbitol, EDTA, combined drug, xylometazoline and BAC groups respectively (Table 2, Figure 1d). Bronchial smooth muscle hypertrophy was detected 70% and 62.5% of BAC and EDTA group respectively (Table 2) (Figure 1e). When evaluated in terms of bronchial lymphoid follicle increase, a significantly greater increase was observed in BAC group than the other groups (60% of rats revealed increased lymphoid follicles) (Table 2, Figure 1f). The number of goblet cells in the upper respiratory tract was determined by histopathological evaluation and compared between the study groups (Table 3).

According to Kruskal-Wallis test, there was significant difference in the mean values of goblet cells between the groups (p=0.000<0.001) (Table 3). According to Post-hoc analysis, difference was originated from sorbitol-combined drug (p=0.008), sorbitol-EDTA (p=0.000), sorbitol-BAC (p=0.000), control-BAC (p=0.005) and xylometazoline-BAC (p=0.035) groups.

DISCUSSION

Sympathomimetic agents such as pseudoephedrine, phenylephrine, oxymetazoline and xylometazoline are frequently used to relieve nasal congestion. Xylometazoline is an alpha (α)-agonist, a sympathomimetic agent which causes adrenergic activation (2, 3). The drug locally constricts blood vessels in the nasal mucosa and relieves nasal congestion, reduces airway resistance and facilitates drainage (4). Although it is thought that some harmful side effects of these drugs and preservatives might be seen, it is not clear even if they are harmful for

Table 1 Frequencies and percentages according to the groups (n=8-10)

	Inflammation of trachea Frequency (Percent)	Bronchial lymphoid follicule Frequency (Percent)	Bronchial edema Frequency (Percent)	Type 2 pneumocyte hyperplasia Frequency (Percent)	Desquamation of trachea Frequency (Percent)	Bronchial smooth muscle hypertrophy Frequency (Percent)
No effect	18 (32.1)	35 (62.5)	26 (46.4)	32 (57.1)	32 (57.1)	39 (69.6)
Mild	19 (33.9)	5 (8.9)	20 (35.7)	12 (21.4)	17 (30.4)	15 (26.8)
Moderate	10 (17.9)	8 (14.3)	9 (16.1)	10 (17.9)	4 (7.1)	2 (3.6)
Severe	9 (16.1)	7 (12.5)	1 (1.8)	2 (3.6)	3 (5.4)	0 (0)
Missing	-	1 (1.8)	-	-	-	-
Total	56 (100.0)	56 (100.0)	56 (100.0)	56 (100.0)	56 (100.0)	56 (100.0)

Table 2 The number of rats that have abnormal histological findings

		Control	Sorbitol	Xylometazolin	BAC	EDTA	Combined
Chronic airway inflammation	No effect	8	4	2	0	0	4
	Mild	0	4	4	4	1	6
	Moderate	0	2	3	2	3	0
	Severe	0	0	1	4	4	0
Bronchial lymphoid follicles	No effect	8	6	6	4	4	8
	Mild	0	2	0	2	1	0
	Moderate	0	1	3	0	2	2
	Severe	0	1	1	4	1	0
Bronchialedema	No effect	8	2	3	2	2	9
	Mild	0	6	5	6	3	0
	Moderate	0	2	2	2	3	0
	Severe	0	0	0	0	0	1
Type 2 pneumocyte increase	No effect	8	0	7	2	7	8
	Mild	0	6	2	3	0	1
	Moderate	0	3	1	4	1	1
	Severe	0	1	0	1	0	0
Desquamation of tracheal epithelium	No effect	8	4	7	7	1	5
	Mild	0	4	3	2	5	3
	Moderate	0	1	0	0	1	2
	Severe	0	1	0	1	1	0
Bronchial smooth muscle hypertrophy	No effect	8	8	9	3	3	8
	Mild	0	1	1	6	5	2
	Moderate	0	1	0	1	0	0
	Severe	0	0	0	0	0	0

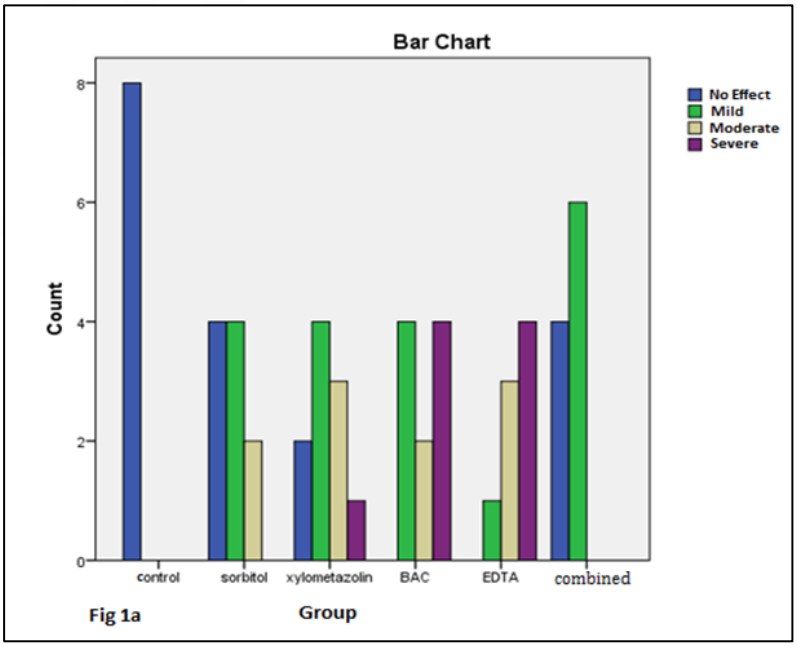


Fig 1a Inflammation of trachea

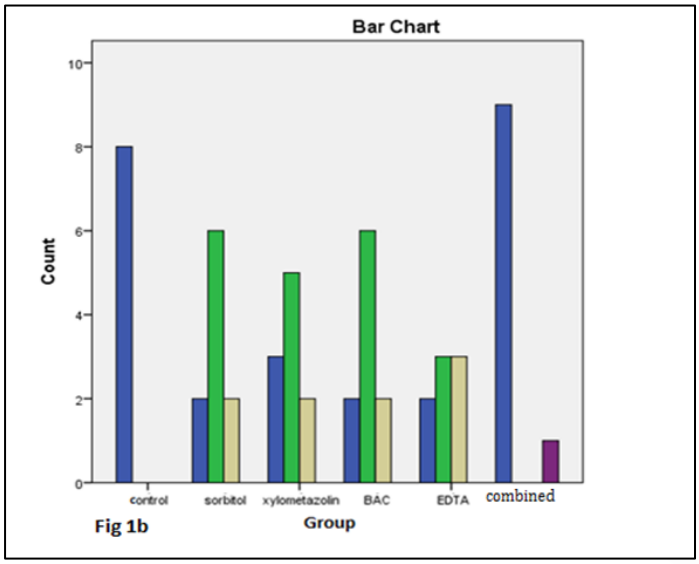


Fig 1b Bronchial edema

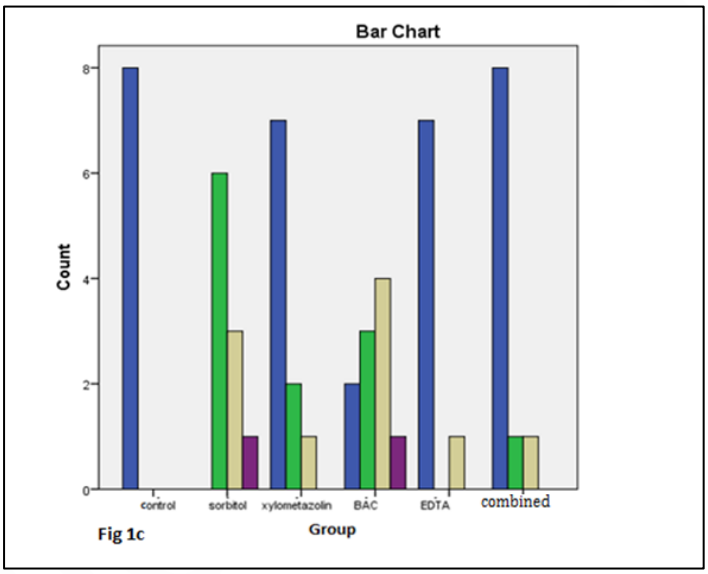


Fig 1c Type 2 Pneumocyte number

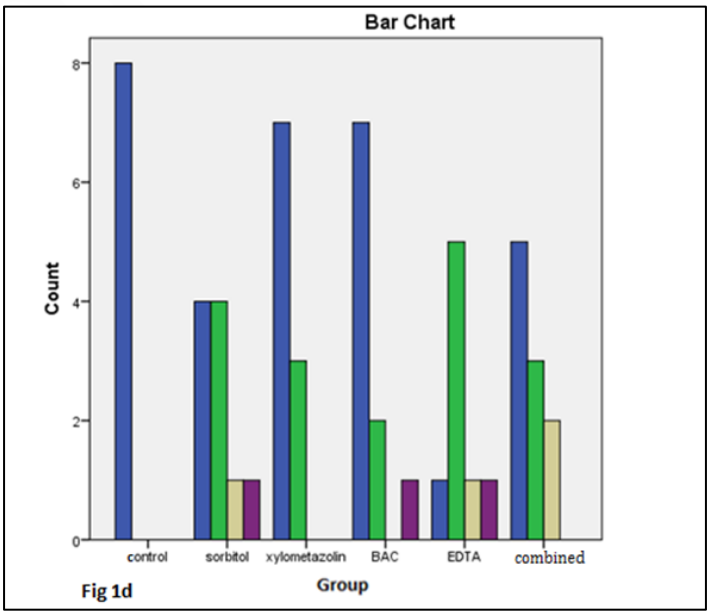


Fig 1d Desquamation of tracheobronchial epithelia

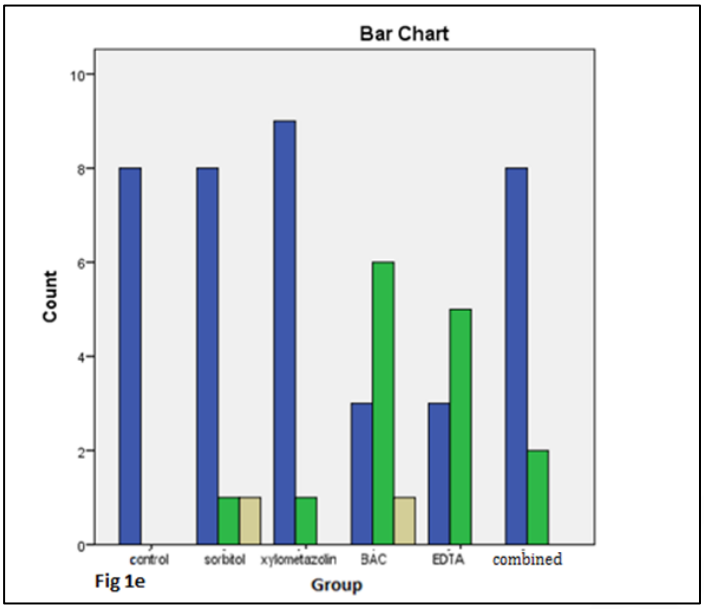


Fig 1e Bronchial smooth muscle hypertrophy

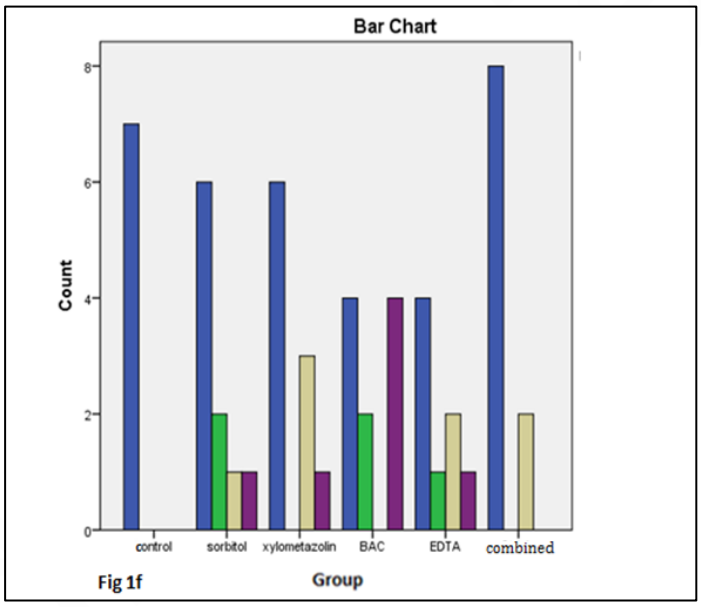


Fig 1f Bronchial lymphoid follicles

Table 3 Variance analysis with Kruskal-Wallis Test among groups according to the number of goblet cells

Groups	N	Mean ± SD	Median (min-max)	P
1 (control)	8	8.38 ± 2.20	8.0 (5-12)	
2 (sorbitol)	10	5.70 ± 1.33	5.5 (4-8)	
3 (xylometazolin)	10	9.70 ± 3.77	10.0 (4-15)	**0.000***
4 (BAC)	10	16.90 ± 3.92	17.0 (10-22)	
5 (EDTA)	8	15.25 ± 5.25	14.0 (9-25)	
6 (Combineddrug)	10	12.10 ± 1.66	12.0 (10-15)	
Total	56	11.30 ± 5.02	11.0 (4-25)	

the lower respiratory tract. It is also unclear whether these drugs are associated with inflammation in asthma exacerbations. According to Eccles et al. (5) adverse event frequency due to intranasal xylometazoline were not significantly higher than placebo. The most frequently seen adverse effects were headache and dysmenorrhea (5). According to a review, (6) although side effects were seen more often in drug-treated patients, the side effects were mostly not severe, and almost all studies concluded that xylometazoline was well tolerated. Patients should be recommended to use a product containing xylometazoline (or other nasal decongestant drugs) at the correct dosage and for a maximum of 7 days to minimize the possibility of rebound congestion. However, especially in atopic-allergic patients, these drugs are used for a longer period of time. Excessive or long-term use of xylometazoline has been known to cause rebound congestion at upper airways (7). Vasoconstrictor α-adrenoceptor agonists are used as nasal mucosa decongestants (8, 9). In a rabbit model, oxymetazoline and

phenylephrine used as nasal spray; caused ciliary loss, inflammatory reaction, secondary bacterial infection and epithelial ulceration (10, 11). Nasal decongestant drugs have different durations of action; which is more than 10 hours for xylometazoline(8). During chronic usage, effect on cell lining of mucous membrane is inevitable. In daily routine, the patients mostly use decongestant drugs as a self-medication. Therefore, we planned this study for long term use of the medicines to reveal better understanding of side effects. Indeed, in our study, tracheal inflammation and bronchial edema were more commonly seen in xylometazoline group; which is consistent with literature.

The use of preservatives and adjuvants in intranasal medications may be harmful in combination with certain drugs and can exacerbate the risk of rhinitis medicamentosa with prolonged use of topical vasoconstrictors (12, 13). Preservatives are also known to decrease ciliary movement and interfere with the self-cleaning capacity of the nose, which may be harmful in chronic nasal medication (14). In our study, findings of upper respiratory tract inflammation were more common in BAC, EDTA and sorbitol groups. Increase of lymphoid follicles at bronchi was more common in BAC and EDTA group compared to the others. Bronchial edema was more common at sorbitol, xylometazoline, BAC and EDTA groups than the control group.

Type II pneumocytes synthesize alveolar surfactant; which has important role in normal function and inflammation of the lung. Surfactant also modulates innate host defense. Any abnormality of surfactant might be one of the mechanisms for the pathogenesis of COPD(15). These Type II cells are the progenitors of Type I cells; they proliferate and differentiate into Type I cells after damage for any reason. It is a part of the repair process of damaged alveolar epithelial barrier. Type II cells also secrete lots of cytokines that can modify the inflammatory and oxidative stress response which may have a role in the COPD pathogenesis (16). In our study sorbitol and BAC group showed increased type 2 cells. That might be a clue for the role of local decongestants at the course of COPD and suggest the requirement of safer preservatives and adjuvants in pharmaceutical forms.

Goblet cells are found at bronchial epithelium. Approximately 25% of the epithelial surface is composed of goblet cells. Main function of goblet cells is mucin secretion into the airway. By the way, mucin, other proteins and lipids produces a thin layer (mucus) in the airway which lubricates the airway surface. Goblet cell hyperplasia is one of the characteristic histopathological findings seen in asthma, and can be diminished by several therapeutic interventions (17, 23). Epithelial cells proliferate and differentiate into goblet cells. Several cytokine signaling pathways induce goblet cell hyperplasia in asthma (18). In several studies, asthmatic mice which had been sensitized by ovalbumine had higher numbers of goblet cells in their BAL fluid than the control group (19, 20). In our study, it was observed that the amount of goblet cells was increased in BAC, EDTA and combined drug groups. According to these findings, local decongestants might be the usual suspects for disease progression in asthma and atopic patients.

Foreign particles are captured all over the bronchi; however, the last place for this process is respiratory bronchioles before alveolar space. There may be some adaptive immune responses such as the disappearance of mucus-secreting cells in respiratory bronchiols; however, cigarette smokers might still have goblet cells. In a murine model, goblet cell hyperplasia, eosinophilic inflammation and bronchial reactivity were found together with asthma (21). In some studies, toxic oxygen radicals have been found to expose their effects by damaging biomolecules, enzymes, membranes, proteins and lipids (22, 24). It might be one of the reasons for the results we found. According to our results, both ingredients and combined drug itself have effects such as increased airway inflammation, edema, type 2 pneumocyte numbers and epithelial desquamation mostly.

CONCLUSION

Although decongestant use has some effects on trachea and bronchial structures, it may be inadequate to arrive at the conclusion that the course of COPD and asthma may be affected by nasal congestion medications. Clinical trials and further animal models are needed to reveal the potential adverse effects of excessive usage of nasal decongestants. Regulation of over the counter medication use should be revised.

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Authors' contributions
Sertaç Arslan: study conception and design, acquisition of data, analysis and interpretation of data, drafting of manuscript, critical revision
Güven Güney: acquisition of data, analysis and interpretation of data, critical revision
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Emre Demir: acquisition of data, analysis and interpretation of data, critical revision
Buket Demirci: study conception and design, acquisition of data, analysis and interpretation of data, drafting of manuscript, critical revision

Conflict of Interests
There is no any funding or any conflict of interests for the study. All of the authors declare that they contribute in every stage of the study and have no any conflicts.

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