FULL ARTICLE

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Probiotics supplementation reduces cigarette smoke-induced damage in the respiratory micro-architecture of mice

Suplementação com probióticos reduz os danos causados pela fumaça de cigarro na microarquitetura respiratória de camundongos

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ABSTRACT

This study evaluated the protective efficacy of probiotics supplementation against cigarette smoke-induced lung emphysema, inflammation, and loss of cilia in mice. Probiotics are known to promote mucosal tolerance and mitigate respiratory injuries. Twenty-four adult mice were randomly divided into three groups: control (Ctr), cigarette smoke (CS), and cigarette smoke + probiotics (CS+P). Probiotics were given for 7 days before exposure to smoke in the CS+P group. Tissue samples of the trachea (goblet cell count and index, loss of cilia), lungs (airspace distention), and bronchoalveolar lavage fluid (BALF) were collected and processed. The results showed a significant increase in acidic and neutral goblet cells in the CS group compared to the Ctr and CS+P groups ($P < 0.05$). Overall, goblet cell number and index were lower in the CS+P group (41.71 ± 5.76, 0.67 ± 0.073) than CS group (56.28 ± 5.34, 1.31 ± 0.28). Inflammatory cells and loss of cilia significantly decreased in mice fed probiotics before exposure to cigarette smoke ($P < 0.05$). Lung emphysema was also significantly reduced in the CS+P group compared to the CS group ($P < 0.05$). In conclusion, dietary supplementation of probiotics reduced lung emphysema, inflammatory cells, goblet cell index, and loss of cilia under conditions of cigarette smoke exposure in mice.

Keywords: Emphysema. Respiratory epithelium. Asthma. Goblet cells. Lungs.

RESUMO

O presente trabalho avaliou a eficácia da suplementação com probióticos contra a indução de enfisema, inflamação e perda de cílios por fumaça de cigarro em camundongos. Sabe-se que os probióticos promovem a tolerância da mucosa e mitigam as injúrias respiratórias. Vinte e quatro camundongos foram divididos, ao acaso, em três grupos: controle (CTR), fumaça de cigarro (CS) e fumaça de cigarro + probióticos (CS+P). Os probióticos foram fornecidos por sete dias antes da exposição à fumaça no grupo CS+P. Foram colhidas e processadas amostras de tecidos da traqueia (contagem de células caliciformes e index, perda de cílios), pulmões (distensão do espaço aéreo) e fluido de lavagem broncoalveolar. Os resultados obtidos revelaram um aumento significativo em células caliciformes acidificadas e neutralizadas no grupo CS quando comparado aos grupos Ctr e CS+P (P<0,05). Os números global e o índice de células caliciformes foram menores no grupo CS+P (41,71+ 5,76; 0,67+ 0,073) que no grupo CS (56,28+ 5,34 e 1,131+ 0,28). As células inflamatórias e a perda de cílios decresceram em camundongos alimentados com probióticos antes da exposição à fumaça de cigarro (P<0,05). O enfisema pulmonar também foi significativamente reduzido no grupo CS+P quando comparado ao grupo CS (P<0,05). A conclusão obtida foi que o fornecimento de dieta suplementada com probióticos reduziu o enfisema pulmonar, as células inflamatórias, o índice de células caliciformes e a perda de cílios nos camundongos expostos à fumaça de cigarro.

Palavras-chave: Enfisema. Epitélio respiratório. Asma. Células cálice. Pulmões.

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Introduction

Cigarette smoke contains more than 5000-7000 carcinogenic and non-carcinogenic hydrocarbons (Nagamma et al., 2011), including oxidants and pro-oxidants (Arinola et al., 2011). The reactive species, especially nitric oxide, is present in the gas phase, and the tar phase contains reactive and nitrogen species; all these chemical substances trigger severe respiratory distress (MacNee & Rahman 2001). It is also believed that almost 10 reactive oxygen species (superoxide, hydrogen peroxide, hydroxyl, and peroxyl radicals) are generated in a single puff of cigarette smoke, causing extensive respiratory irritation (Nagamma et al., 2011).

The level of irritation or inflammation in the airways of smokers is crucial for the development and progression of the disease. Exposure to cigarette smoke causes regional distention of airspaces near terminal bronchioles (Biselli et al., 2011), resulting in histological emphysema (Podowski et al., 2012). Smokers' lung parenchyma is regularly exposed to repeated injuries (Simet et al., 2010) and various mechanisms of cell repair function at molecular and cellular levels, such as squamous cell metaplasia and goblet cell hyperplasia (Rennard, 1999). Several studies have demonstrated that probiotics (*Bifidobacterium lactis, Enterococcus faecium AL41*, *Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus paracasei*) improve various body systems of diseased animal models (Arunachalam et al., 2000; Forsythe et al., 2007; Luppi et al., 2005; Wang et al., 2017). Probiotics are beneficial bacteria that confer health benefits to the host in the form of reduced emphysema and cilia loss in the respiratory tract. These nutritional agents modulate the host defense against respiratory infections through competitive interactions with pathogens or inhibition of epithelial adherence by pathogens (Tannock et al., 2000) and, thus, protect the body from allergic reactions and chronic inflammation. Since probiotics have the potential to scale down the deterioration of respiratory architecture due to cigarette smoke, this study was designed to evaluate the effect of prophylactic use of probiotics on cigarette smoke-induced respiratory damage.

Materials and Methods

Twenty-four adult BALB/c male mice weighing 20 ± 3 g were used in this study. Animals were maintained in a controlled room at a temperature of 21-23 °C, and a 12-h light/12-h dark cycle with free access to food and water was provided. Mice were acclimatized for one week, and the total duration of the experiment, including the acclimatization period, was 35 days. The research was conducted in the Department of Anatomy, University of Veterinary and Animal Science, Lahore, Pakistan, in compliance with the UVAS Institutional Guidelines for the care and use of experimental animals no. DR/ 377, Vide. No. 27-8-2014.

Experimental design

Mice were randomly divided into three groups (n=8 mice per cage), i.e., Ctr (Control; exposed to air only), CS (cigarette smoke; mice exposed to the smoke of 6 cigarettes/day for 6 days/week for 4 weeks), and CS-P (exposed to cigarette smoke + Probiotics). Multi-strain commercial preparation of probiotics (Protexin of Hilton Pharma Pvt. Limited) was used in this study (Table 1).

Probiotics were orally given 1 g per kg/day of pelleted grains feed during the first seven days of the experiment. Six cigarettes were smoked for 2 h, 6 days/week for 4 weeks. Mice were exposed to smoke via one inlet and two outlets of the specially designed inhalation cage (40 cm long, 30 cm wide, and 25 cm high) (Vasconcelos et al., 2019).

Table 1 – Composition (100g) of Protexin™, Hilton Pharmaceuticals, Private, Limited-Pakistan

Composition	Concentration
Lactobacillus plantarum	1.89×10^7 cfu/g
Lactobacillus delbrueckii subsp. *Bulgaricus*	3.09×10^7 cfu/g
Lactobacillus acidophilus	3.09×10^7 cfu/g
Lactobacillus rhamnosus	3.09×10^7 cfu/g
Bifidobacterium bifidum	3.0×10^7 cfu/g
Streptococcus salivarius subsp. *Thermophilus*	6.15×10^7 cfu/g
Enterococcus faecium	8.85×10^7 cfu/g
Aspergillus oryza	7.98×10^7 cfu/g
Candida pintolopesii	7.98×10^7 cfu/g

Sample collection

At the end of the experiment (24 h after the last exposure to smoke), seven mice from each group were randomly selected for sampling. After anesthesia with Ketamine-Xylazine (200 mg/kg + 10 mg/kg, i.m., respectively), tracheal lavage was performed, and tissue samples were aseptically collected after the euthanasia (chloroform). Briefly, the lungs and trachea were dissected. Tracheas were removed and maintained in a closed, sterile, 15-ml conical tube containing penicillin and streptomycin at room temperature until processing. Tissue samples were processed through the paraffin embedding technique of Hematoxylin & Eosin (H & E) as well as combined Alcian blue/Periodic acid-Schiff (AB-PAS) stain (Bancroft & Gamble, 2008). The H & E-stained slides were used to observe tracheal cilia and lung airspaces while combined Alcian blue/Periodic acid-Schiff (AB-PAS) stained tracheal tissues were observed for goblet cell count. All slides were examined under a light microscope (LABOMED-USA).

Histochemical characterization of goblet cells and cell index

Combined Alcian blue/Periodic Acid Schiff (PAS) Staining for goblet cells was performed. Goblet cells containing acidic glycoproteins were stained blue, neutral glycoproteins were stained magenta, and mixed goblet cells containing both acidic and neutral glycoproteins were stained purple (Bancroft & Gamble, 2008). Only the cells containing well-developed granules were considered representative of the positive reaction. The ratio of goblet cells to epithelial cells was obtained by counting the interspersed goblet cells/ 100 epithelial cells in a section. Positively stained cells reaching the luminal surface were also calculated.

Loss of cilia

The loss of cilia was observed in five fields per section at 40X under a light microscope. The number of cells with or without cilia was counted on each slide, and percentages were calculated. The data were collected after estimating the rate of cells without ciliated projections in all the groups separately.

Lung morphometry

To perform lung morphometry, lungs were fixed, and morphometric measurements were made on hematoxylin and eosin-stained lung sections. Five concentric semicircles of 50, 100, 150, 200, and 250 μm radii were drawn with terminal bronchiole in the center. The radius of each semicircle was divided by the number of intersections (alveoli) on the line (Biselli et al., 2011), which is also known as the mean linear intercept (Lm). A minimum of five different fields per slide were observed in a blinded manner. Morphometry was performed using software (ProgRes@ 2.1.1 Capture Prog Camera Control Software JENOPTIK). The fewer intersections/semicircles, the greater the airspace enlargement.

Bronchoalveolar lavage (BAL)

To perform the lavage, three mice were randomly selected from each group. After exposing the trachea, both lungs were washed with 2ml PBS through a 24-gauge cannula. The BAL fluid was aspirated and centrifuged at 1500 rpm for 2 minutes at 4° C. The supernatant was discarded, and 100 μl PBS was added to the pellet (Yu et al., 2010). Differential cell count was performed by staining slides with Wright-Giemsa stain and counting at least 200 cells per slide under a light microscope.

Statistical analysis

Statistical analysis was conducted with the Statistical Package for Social Science (SPSS) for window version 21 (Chicago, IL, USA). The data were analyzed through one-way ANOVA, and means were compared using Duncan's multiple-range test. The differences were considered significant at (P <0.05), and results were expressed as means ± standard error (SEM).

Results

Goblet cell hyperplasia in the respiratory airway indicates illness, and our findings showed a significant shift in goblet cell number in the CS group compared to other groups. Acidic goblet cells (AGC), neutral goblet cells (NGC), and mixed goblet cells (MGC) in the CS group were significantly higher (27.71 ± 3.40, 6.85 ± 1.34, 4.71 ± 1.49). The goblet cell index was also significantly higher (P < 0.01) in the CS than in the Ctr and CS+P groups (Table 2, Figure 1). The comparative reduction in goblet cell index in CS+P shows adequate protection against cigarette smoke. A complete loss of cilia was observed in the tracheal epithelium in the CS group. On the other hand, the appearance of ciliated epithelium in the CS-Pro group was nearly like that of the Ctr group (Figure 2).

In the control group, the tracheal epithelium was expected with the presence of cilia. However, in the CS group, the ciliated epithelium of the trachea was thoroughly damaged by smoke exposure, and loss of cilia was significantly reduced in the CS+P group (P < 0.05) compared with the CS group (Figure 2).

Table 2 – Effect of probiotics on the GC count, type of mucin, and GC index in the tracheal epithelium (Mean ± SEM)

	Ctr (n = 7)	CS (n = 7)	CS-Pro (n = 7)	*P*-value
GC	27.00 ± 3.4 [c]	56.28 ± 5.34 [a]	41.71 ± 5.76 [b]	0.00<
AGC	6.00 ± 1.63 [c]	27.71 ± 3.40 [a]	11.57 ± 2.22 [b]	0.00<
NGC	2.42 ± 0.97 [c]	6.85 ± 1.34 [a]	4.00 ± 1.15 [b]	0.00<
MGC	1.28 ± 0.75 [c]	4.71 ± 1.49 [a]	2.71 ± 0.75 [b]	0.00<
EC	73.00 ± 3.4 [a]	43.71 ± 5.34 [c]	59.71 ± 2.56 [b]	0.00<
GC:EC	0.36 ± 0.066 [c]	1.31 ± 0.28 [a]	0.67 ± 0.073 [b]	0.00 <

Means within each grouping with different letter designations differ significantly ($P < 0.05$). n: number of animals; SEM: standard error of mean, AGC= Acidic Goblet Cells; NGC= Neutral Goblet Cells; MGC= Mixed Goblet Cells; GC= Goblet Cell; EC= Epithelial Cells, GC:EC= GC index.

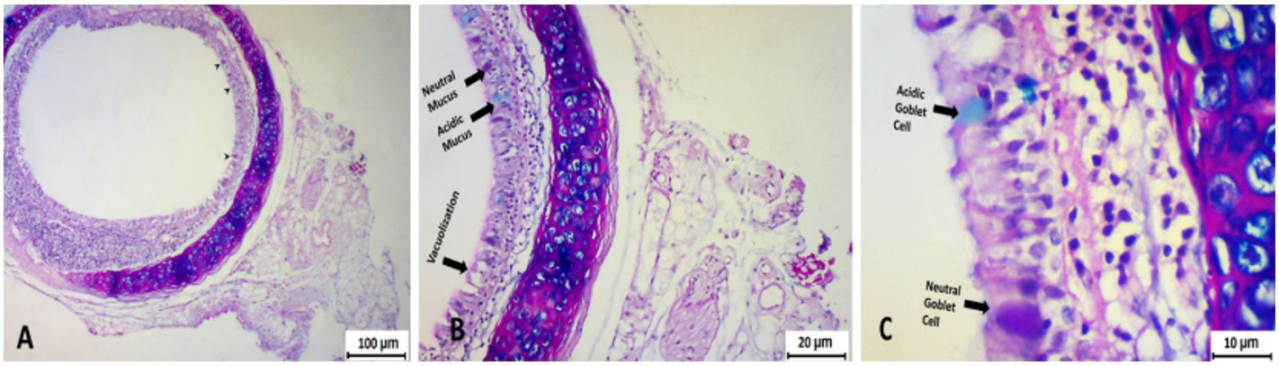

Figure 1 – PAS-AB-stained histological sections at various magnifications of the objective lens of the control group (A) Microscopic field at 4X shows the tracheal section in mice (arrowheads), (B) At 10X differently stained goblet cells are visible, (C) At 40X acidic goblet cell (Blue) and neutral goblet cell (Magenta).

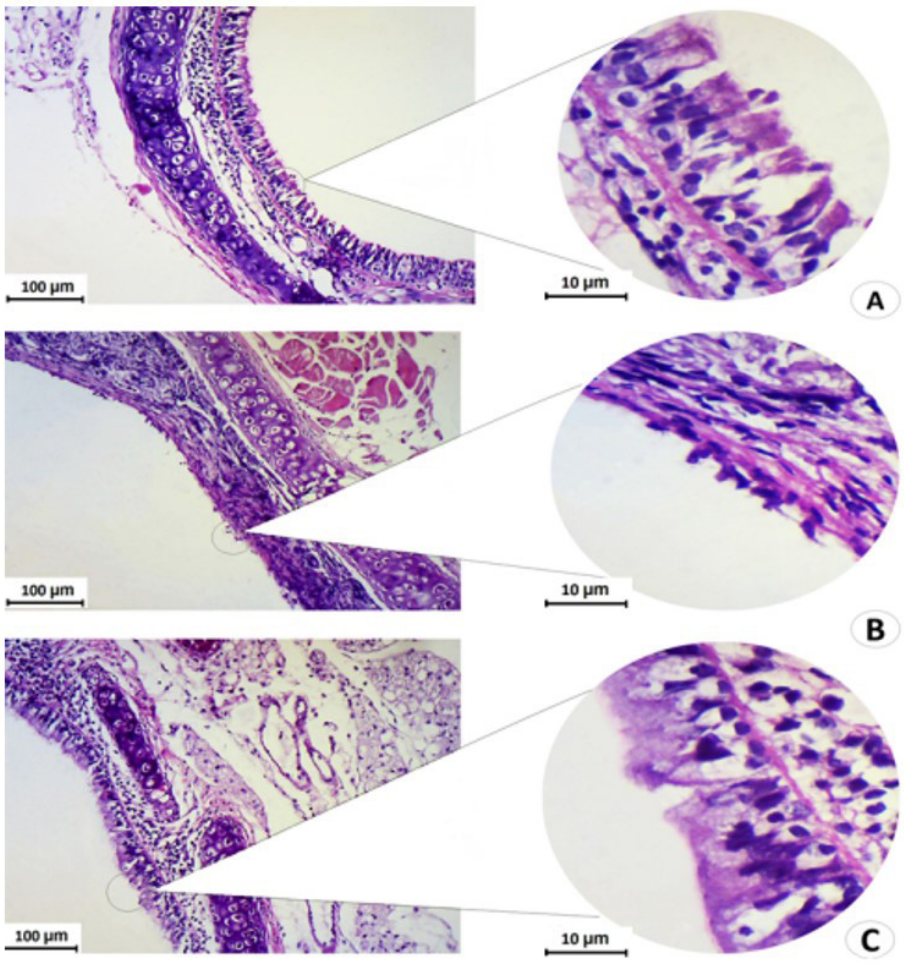

Figure 2 – Histologic sections represent mouse tracheae of the control group (A) and after exposure to cigarette smoke (B) and probiotics group (C) (magnifications, ×10 and ×40). Mice exposed to cigarette smoke for 28 days retained a pseudostratified columnar ciliated epithelial layer of cells like that in control mice (A, C). Mice exposed to smoke for 28 days have lost most of their ciliated cells (B).

In the Ctr group, the air space distension was within the normal parameters of lung morphometry, i.e., the absence of collapsed or distended alveoli near the terminal bronchiole. On the contrary, the highest enlargement of airspaces was found in the CS group as compared with the Ctr and CS-Pro groups ($P < 0.05$) (Figures 3, 4).

It was also observed that out of five concentric semicircles, lung emphysema was primarily seen at a 150 µm distance in all samples of the CS group. There was no marked enlargement of air spaces in CS+P compared to the CS group (Figures 4, 5).

The bronchoalveolar lavage (BAL fluid) findings revealed a higher percentage of inflammatory cells (neutrophil, eosinophil, macrophage, erythrocyte, basophil, and monocyte) in the CS exposed animals' group ($P < 0.05$) and especially neutrophils and eosinophils were higher (Table 3, Figure 6).

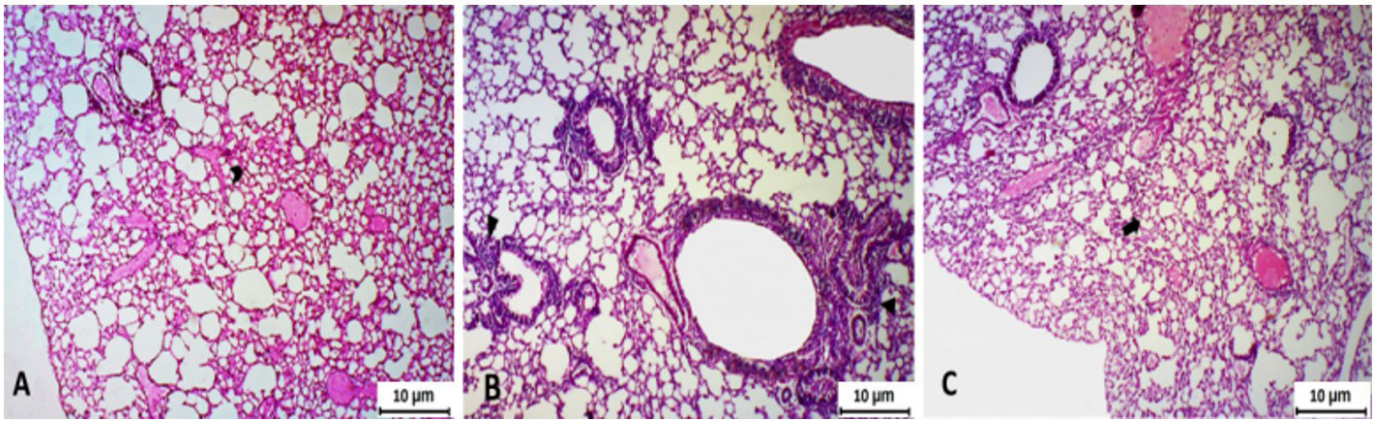

Figure 3 – (A) Histological sections represent the normal section of lungs and air spaces (arrowhead), (B) shows cigarette smoke-exposed section of lung and infiltration of polymorphonuclear cells at various sites (arrowheads) and damage to air spaces (C) Figure shows the areas of recovery (arrow) from cigarette smoke and lower infiltration of polymorphonuclear cells in the probiotic supplemented group.

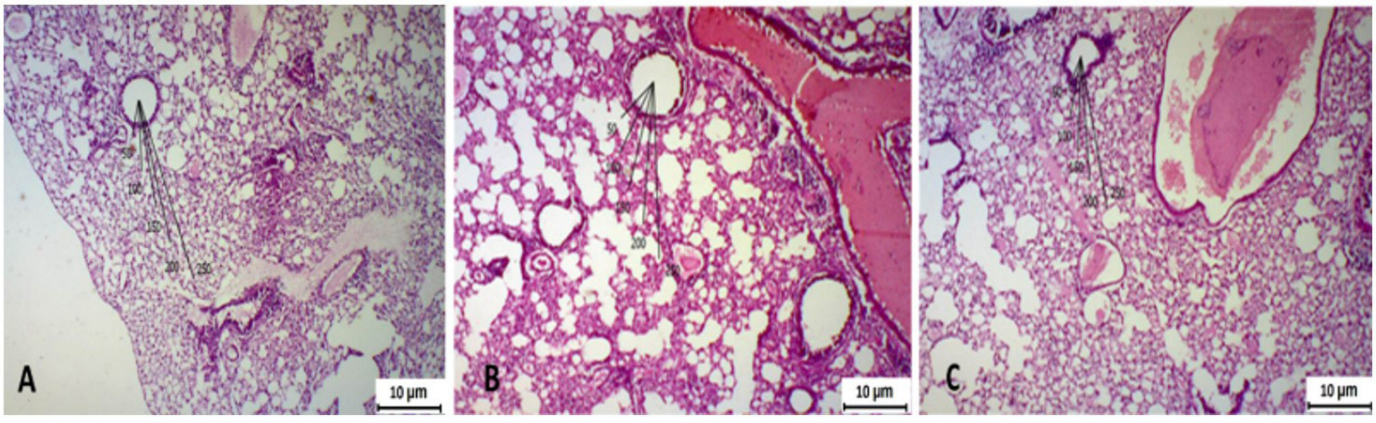

Figure 4 – Shows lung morphometry and measurement of air spaces at different radii from the center of the terminal bronchioles (A) Control group (B) Cigarette smoke group (C) Probiotic group.

Table 3 – Effect of probiotics on cellular composition (%) of BALF (Mean± SEM). Different superscripts represent the level of significance ($P < 0.05$)

	Ctr (n = 3)	CS (n = 3)	CS-Pro (n = 3)	*P-value*
NU	1.00 ± 1.00^{c}	20.33 ± 1.52^{a}	16.00 ± 9.5^{b}	0.01
EO	0.66 ± 0.57 ^{c}	14.66 ± 0.57 ^{a}	6.33 ± 0.57 ^{b}	0.01
MC	0.66 ± 0.57 ^{c}	27.66 ± 3.05 ^{a}	13.66 ± 3.78 ^{b}	0.01
LY	0.00 ± 0.00 ^{c}	03.66 ± 1.15 ^{a}	1.33 ± 1.15 ^{b}	0.09
ER	1.00 ± 0.00 ^{c}	07.00 ± 1.00 ^{a}	2.00 ± 1.00 ^{b}	0.01
BS	0.00 ± 0.00 ^{c}	1.33 ± 1.15 ^{a}	0.33 ± 0.57 ^{b}	0.15
MO	0.00 ± 0.00 ^{c}	1.00 ± 1.00 ^{a}	0.00 ± 0.00 ^{b}	0.13
EC	96.33 ± 2.51 ^{c}	24.66 ± 8.73 ^{a}	67.00 ± 8.00 ^{b}	0.01

$^{a, c}$ Means within each grouping with different letter designations differ significantly ($P < 0.05$). n: number of animals; SEM: standard error of mean and NU= Neutrophil; EO= Eosinophil; MC= Macrophage; LY= Lymphocyte; ER= Erythrocyte; BS= Basophil; MO= Monocyte; EC= Epithelial cells)

Inflammatory cells were lowest ($P < 0.05$) in the Ctr group after 28 days of air exposure, but epithelial cells were highest ($P < 0.05$). Interestingly, reduced inflammatory cells were seen in CS+P than in the CS group ($P < 0.05$), and the differences were pronounced even after exposure to cigarette smoke.

Discussion

The present study demonstrated that lung emphysema and goblet cells increased in the CS group after four weeks of smoke exposure compared to the control group. Goblet cell metaplasia is a common manifestation of respiratory disorder. Cigarette smoke stimulates the proliferation of tracheal epithelial cells, especially goblet cells (Luppi et al., 2005). Similarly, Komori et al. (2001) reported goblet cell hyperplasia of tracheal epithelium even after 14 days of cigarette smoke exposure in the guinea pig. Previous data suggest that short-term exposure to cigarette smoke can cause goblet cell hyperplasia and a reduction in ciliated cells (Biselli et al., 2011). Although the goblet cell number significantly increased in the CS+P group compared to the Ctr group, it also reduced considerably compared with the CS group. Probiotics act as immunomodulatory agents. Therefore, in this prophylactic study, a dose was given during the first week of the experiment to activate the host defense pathways.

The general mechanism of the epithelium is to protect mucosa against infections by producing more acidic types of mucins. Cigarette smoke also causes a shift in the production of glycoproteins by the goblet cells in mice. A high ratio of goblet cells to epithelial cells ($P >0.05$) in the CS group also indicates the increased mucosal challenge to the respiratory system in mice. This goblet cell hyperplasia for excessive mucous production propels debris and pathogens towards the pharynx, ultimately out of the body. In our study, goblet cells with acidic mucins were higher in the CS group, which agrees with Ardite et al. (2006) findings, which illustrated that tobacco smoke induces hyperplasia of Alcian-blue positive goblet cells and hypoplasia of periodic acid Schiff-positive cells. Goblet cell hyperplasia acts as a defense mechanism of the respiratory system, thus cleaning the air passage from irritating cigarette substances. However, the probiotic supplementation decreased goblet cell numbers, lowering mucus production. These dietary additives not only enhance immunity but also the mucosal tolerance is increased, especially in cases of respiratory distress (Yu et al., 2010). This argument was supported by the results of reduced goblet cell index in the CS+P group ($P >0.05$).

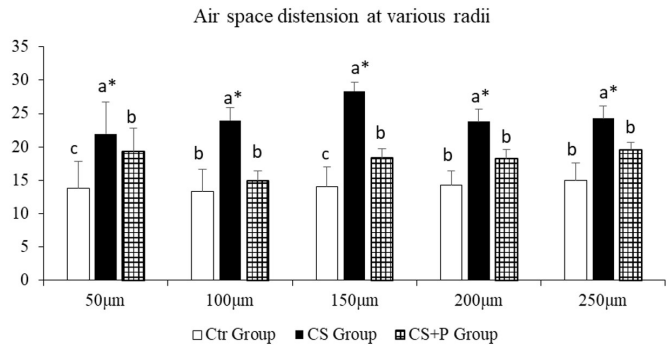

Figure 5 – Shows the enlargement of airspaces due to cigarette smoke at various radii. (Ctr) Control group (CS) Cigarette smoke group (CS+P) Probiotic group, showing measurement of air spaces at different radii from the center of the terminal bronchioles. The letters a, b, c, are showing extent of damage in the air spaces of lungs as "a-maximum", "b-medium", "c- minimum" enlargement and esterik (*) indicates pronounced damage in CS group.

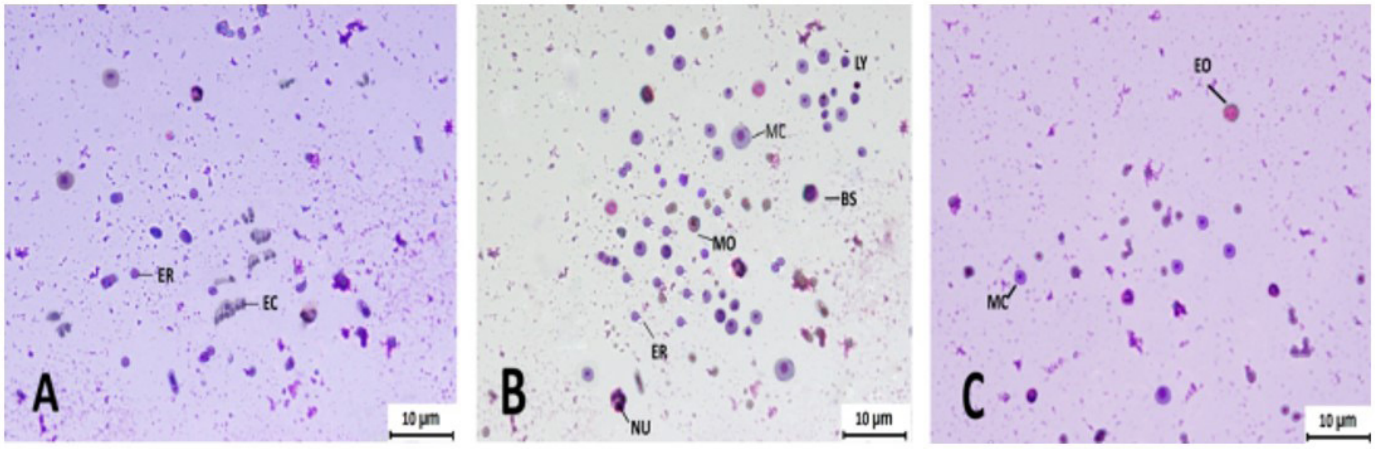

Figure 6 – Representative pictures of cell composition of bronchoalveolar lavage (A) Control group: Various cells ER (erythrocytes), EC (epithelial cells) are shown (B), Cigarette smoke group: MO (monocytes), MC (macrophages), BS (basophils), LY (lymphocytes), NU (neutrophils), (C) Probiotic group: MC (macrophages), EO (eosinophils). Images are taken at 40X.

In the present study, CS and CS+P groups showed loss of cilia due to chemicals and smoke toxins. During respiratory infections, the ciliated epithelium serves as the first line of defense, and long-term cigarette smoke exposure directly damages these cilia (Simet et al., 2010). That is why the complete loss of cilia was seen in the CS group. The irritating substances and chemical compounds in smoke damage the protective coating of respiratory epithelium and cilia. Işik et al. (2004) reported significant differences in the tracheal epithelium of the smoke-exposed rats after 60 days of exposure. Shedding of cilia has also been observed over a year-long exposure of tracheal epithelium to cigarette smoke (Simet et al., 2010). This decreased area of ciliated cells in the respiratory epithelium is a critical factor for COPD development, resulting in reduced lung function (Sun et al., 2017).

It is essential to mention that the CS+P group also suffered significantly higher cilia loss than the Ctr group ($P > 0.05$). However, this loss was lower ($P < 0.05$) than the CS group. This protective role of probiotics for tracheal epithelium may be attributed to the healthier immune system of the host, as reported earlier that COPD can be avoided using probiotics (Mortaz et al., 2015). Initial observation of this study also revealed the protective effect of probiotics against loss of cilia due to cigarette smoke. Thus, our findings of probiotic supplementation in the smoke-exposed group describe the supporting results in mice lung health.

The mucous (goblet) and serous cells (ciliated) are primarily seen in the epithelium of proximal airways. In contrast, the Clara cells (non-ciliated) are found in the distal airways (Plopper et al., 1984). The cigarette smoke's effect is not only limited to the upper airways and extends to the lower airways up to the lungs. Valença et al. (2004) stated that a gradual increase in cigarette smoke exposure results in more significant distention of airspaces in mice lungs. Therefore, cigarette smoke damages the lungs in a dose-dependent manner, potentially producing emphysema (Podowski et al., 2012). In this study, cigarette smoke-exposed animals in both groups, CS and CS+P, showed lung emphysema at various levels of radii. The emphysema was very severe in CS and burst or collapsed alveoli were seen. Similarly, Biselli et al. (2011) found that airspace enlargement occurs at 100 μm and 150 μm distance from the terminal bronchiole after two months of exposure to cigarette smoke. The dose of cigarette smoke was kept constant in the present study, but repeated exposure resulted in severe lung damage. In the CS group, highly distended airspaces near the terminal bronchiole were seen at 100 to 200 μm distance ($P > 0.05$). Knudsen et al. (2010) documented that at least 6 months of smoke exposure is required to develop widespread airspace distention. Therefore, in this study, after four weeks of exposure to cigarette smoke, only limited regions developed airspace enlargement, and that was mostly confined at 150 μm. Though heterogeneous air space enlargement was evident in the CS+P group, most areas appeared quite normal, indicating a normalizing of lung architecture and repair.

Despite limitations in the pathophysiology of our model, it is pertinent to mention that 28 days of CS exposure caused a shift in the inflammatory pattern of neutrophils and lymphocytes in the bronchoalveolar lavage fluid of mice. The total cell counts on BAL fluid increased substantially with exposure to smoke in the CS group ($P > 0.05$) and shifted towards an abundant presence of neutrophils, lymphocytes, and macrophages were also seen in both groups, i.e., CS and CS+P. Higher percentages of inflammatory cells were found in BALF using Wright-Giemsa stain (a proven technique for differential cell counts) as described by Baughman & Drent (2001). The composition of BALF showed higher neutrophil and eosinophil counts in the CS group compared to other cells, as cigarette smoke causes increased eosinophilia and neutrophilia in the BAL fluid (Komori et al., 2001). Eosinophilia is mainly observed in the BAL fluid of active smokers, while neutrophilia and an increase in lymphocytes are reported in ex-smokers (Wen et al., 2010). It suggests that chronic passive exposure to cigarette smoke causes changes in BALF composition as affected by active cigarette smoking.

Elliott et al. (2007) also found that alcohol + cigarette smoke increased neutrophils, lymphocytes, and macrophages, which agrees with our study. The pre-treatment with probiotics reduced neutrophil and eosinophil counts in the CS+P group ($P > 0.05$). Wang et al. (2017) and Sun et al. (2017) also reported a protective effect of higher doses of *Lactobacillus paracasei* and *Clostridium butyricum* in asthma models, respectively. *Lactobacillus acidophilus* is also known to provide primary defense against pathogens by inducing a non-specific immune response in mice (Jung et al., 2010).

Probiotics in feed supplements have gained worldwide attention due to their direct beneficial effects on the digestive and immune systems (Jung et al., 2010). The results of this study also indicate a significant impact of probiotics against cigarette smoke-induced respiratory damage in the mice. Previously, only allergic diseases like asthma were thought to be preventable by probiotics, but various studies have reported a wide range of other beneficial effects. Forsythe et al. (2007) discovered that oral administration of *Lactobacillus reuteri* stimulates the immune response in the gastrointestinal tract

and lymphoid organs, suggesting decreased airway hyper-responsiveness and several inflammatory cells in lung tissue of the asthmatic mouse model. Similarly, *Lactobacillus casei rhamnosus* reduces airway inflammation and hyper-reactivity in a mouse model of allergic airway inflammation (Yu et al., 2010), and *Lactobacillus* isolates show anti-campylobacter activity (Dec et al., 2018). It is also stated that *Lactobacillus acidophilus* decreases the eosinophil number in asthma patients (Wheeler et al., 1997). Smith et al. (2016) described the inefficiency of the prophylactic use of probiotics in prescriptions for asthma. Still, Ezendam & van Loveren (2006) suggested that probiotics affect the immune response and enhance mucosal tolerance, likewise in this study. Similarly, Jung et al. (2010) reported that probiotic supplementation improves chickens' immune activity and increases survivability against bacteria. Despite the studies mentioned above, many aspects of the probiotic-host immune system crosstalk are still unknown and need further exploration.

Conclusions

In conclusion, the prophylactic use of probiotics is helpful in the reduction of respiratory-induced damage by exposure to cigarette smoke in mice through modifications of a micro-architecture of the respiratory system.

Conflict of Interest

The authors declare no conflict of interest.

Ethics Statement

The study was conducted in compliance with the UVAS Institutional Guidelines for the care and use of experimental animals no. DR/ 377, Dated: 27-8-2014.

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