Peer Review File

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Reviewer A

This manuscript evaluates the effects of YQGB, a traditional Chinese medicine, on COPD patients and on cultured human bronchial epithelial (HBE) cells treated with cigarette smoke extract. Pulmonary function, mitochondrial potential of peripheral blood lymphocytes, and ROS production, ATP levels, proliferation rate and SIRT5 expression in HBE cells were evaluated. This is an interesting study but this work has significant shortcomings that need to be corrected. In addition, the English must be corrected throughout the manuscript (including the abstract). Some sentences are not clear, leading to misunderstanding of the message.

Running title

"YQGB regulates SIRT5 expression…" and not "TCM regulates SIRT5 expression…"

Reply: Thank you for the comment. Running title has been revised.

Changes in the text: see Page 1, line 3.

Introduction

-Page 3, lane 97: "…by elevating SIRT5 expression…"

Reply: Thank you for the comment. What we meant is that "Yiqigubiao pill can increase the expression of SIRT5" and the description had been revised.

Changes in the text: we have modified our text as advised (see Page 5, line 90-92)

Materials and methods

-Companies and countries must be indicated for all the materials.

Reply: Thank you for the comment. The information had been added.

Changes in the text: we have modified our text as advised (see Page 5, line 95-106)

-Page 4, lane 150: "Ten COPD patients were randomly divided into oral YQGB group (n = 17) and placebo group (n = 17)…": Only 10 patients out of 34 were randomly divided into the groups? Could you explain?

Reply: Thank you for the comment. The sentence has been revised.

Changes in the text: see Page 7, line 140.

-Page 4, lane 151: "The dose of YQGB was 10 pills per dose…": Is it possible to know what is the exact quantity of YQGB administered to patients (in mg or g)?

Reply: Thank you for the comment. Each 10 pills are equivalent to 5g of raw material.
Changes in the text: we have modified our text as advised (see Page 7, line 141)

-Page 4, lane 161: What is the final percentage of FBS in the medium? This should be indicated.

Reply: Thank you for the comment. 10% FBS was added to the medium.

Changes in the text: we have modified our text as advised (see Page 8, line 159)

-Page 4, lane 161:"cyanine chain double antibodies": I am sorry, I don't know what you are talking about. These antibodies are present in the medium? Can you add the company where it was purchased?

Reply: Thank you for the comment. Cyanine chain double antibodies were purchased from Shanghai Zeye Biotechnology Co., LTD., the purpose of adding double resistance is to inhibit bacterial growth and avoid cell pollution.

Changes in the text: we have modified our text as advised (see Page 8, line 160)

-Page 5, lane 184: Is the siRNA Negative Control a scrambled siRNA? This should be indicated.

Reply: Thank you for the comment. The siRNA Negative Control (NC) is a scrambled siRNA.

Changes in the text: we have modified our text as advised (see Page 9, line 183).

-Page 5, lane 198: What is the composition of the lysis buffer? This should be indicated.

Reply: Thank you for the comment. The main ingredients are 50mM Tris (pH 7.40), 150mM NaCl, 1%TritonX-100, 1% sodium deoxycholate, 0.1%SDS, As well as 2mM sodium pyrophosphate, 25mM B-glycerophosphate, 1mM EDTA,1mM Na3VO4, 0.5ug/ml leupeptin and other inhibitors. It can effectively inhibit protein degradation.

Changes in the text: we have modified our text as advised (see Page 10, line 196-199)

-Page 5, lane 203: "After gray analysis of…": Replace with "After densitometry analysis of…".

Reply: Thank you for the comment. The sentence has been revised.

Changes in the text: we have modified our text as advised (see Page 10, line 207)

-Page 6, lane 219: The JC-1 probe is at a final concentration of 2 μM? Exact? This is not clear.

Reply: Thank you for the comment. The cell concentration was adjusted to 1×10^6 cells/ml and then added to a 2 μm JC-1 probe, and the cells were incubated for 30 min at 37°C in the dark.

Changes in the text: we have modified our text as advised (see Page 11, line 218-220)

Results

-Page 6, lane 247: The healthy subjects are not "healthy patients". Please replace with "healthy controls" or "healthy subjects".

Reply: Thank you for the comment. The sentence has been revised.

Changes in the text: we have modified our text as advised (see Page 11, line 252)

-Table 1:

Title: “collected subjects” and not “collected patients”.

Reply: Thank you for the comment. The sentence has been revised.

Changes in the text: we have modified in table 1.

“Male, n (%)” and not “Male Sex, n (%)”.

Reply: Thank you for the comment. It has been revised.

Changes in the text: we have modified in table 1.

c-What is “Smoking history, n (%)”? There is no unit. The numbers are not presented as “n (%)”.

Reply: Thank you for the comment. "n" means the number of smokers. n% represents the percentage of patients with a history of smoking in the group.

Changes in the text: we have modified our text as advised (see Table 1, note) .

d-It should be written “Pack year, mean ± SD” and not “Pack/yr, mean ± SD”.

Reply: Thank you for the comment. It has been revised.

Changes in the text: we have modified in table 1.

e-121.6 pack year for COPD patients and 44 pack year for healthy controls are very high numbers. Are these numbers correct?

Reply: Thank you for the comment. Yes, the number is correct. There were also smokers in enrolled healthy people, but the number of cigarettes smoked was very small. Much of them smoked occasionally or consumed 2-3 cigarettes a day, and their lung function tests were normal.

Changes in the text: Since the number is correct, no change was made in the manuscript regarding to this comment.

f-The values are presented for 17 COPD patients while 34 patients participated to the study. Please, present the results of the 34 COPD patients.

Reply: Thank you for the comment. Baseline data from 34 COPD patients were compared with healthy controls.

Statistical analysis: Age and BMI were measurement data, and the results of normality test showed that their distribution obeyed normality, so they were statistically described using mean ± standard deviation (mean ± SD), and the differences between groups were statistically described using T-test. Counting data such as gender and smoking history were described using

the number of cases (n) and composition ratio (%), and Chi-square test was used to analyze the composition differences between groups. The test level α is set to 0.05.

The results showed that there were 34 COPD patients and 17 healthy controls. The average BMI of COPD patients was 23.4±3.7, lower than that of healthy controls (26.7±3.8), and the difference was statistically significant ($P<0.05$). There were no significant differences in age, sex, ethnicity and occupation between COPD patients and healthy controls ($P>0.05$). There were differences in smoking history, $P<0.05$.

Changes in the text: we have modified our text as advised (see Table 1, see Page 12, line 245-249),

-Table 2:

a-Units for FEV1 and FVC are missing.

Reply: Thank you for the comment. It had been revised.

Changes in the text: we have modified our text as advised (see Table 2)

b-Replace "FEV1%, mean ± SD" with "FEV1 (%predicted), mean ± SD".

Reply: Thank you for the comment. It had been revised.

Changes in the text: we have modified our text as advised (see Table 2)

c-There is a problem with the FEV1 values presented in the table: Page 14: "Patients with COPD who underwent YQGB had significant improvements in lung function at FEV1 and FVC compared with PB (Table 2)". However, Table 2 shows that PB treatment decreases the FEV1 values (from 1.4 to 1.3) as well as the YQGB treatment (from 1.4 to 1.2). Therefore, it seems that there is no improvement of lung function looking at FEV1 values. Could you explain?

Reply: Thank you for the comment.

1. The T test of two independent samples was used in the previous statistical table, which may not be able to completely represent the changes in lung function before and after treatment. Therefore, the comparison between the two groups of patients before and after treatment was conducted again. There were significant differences in FEV1 and FVC before and after treatment in COPD group ($P<0.05$). There was no significant difference in FEV1/FVC% before and after treatment, $P>0.05$; See Table 2 for details.

2. There was no statistical difference in baseline data before treatment and indexes after treatment between YQGB and COPD groups, and indexes after treatment in YQGB group were not higher than those in COPD group, which may be related to the slightly higher pulmonary function indexes in COPD group than YQGB group in baseline data (see table below).

Test Statistics[a]

	FEV1 pt	FEV1 t	FVC pt	FVC t	FEV1/FVC pt	FEV1/FVC t
Mann-Whitney U	132.000	127.500	132.000	132.500	140.000	134.000
Wilcoxon W	285.000	280.500	285.000	285.500	293.000	287.000
Z	-.431	-.586	-.431	-.413	-.155	-.362
Asymp. Sig. (2-tailed)	.667	.558	.667	.679	.877	.718
Exact Sig. [2*(1-tailed Sig.)]	.683[b]	.563[b]	.683[b]	.683[b]	.892[b]	.734[b]

pt: before treatment
t: after treatment
a. Grouping Variable: z group
b. Not corrected for ties

Test Statistics[a]

	FEV1 pt	FEV1 t	FVC pt	FVC t	FEV1FVC pt	FEV1FVC t
Mann-Whitney U	132.000	127.500	132.000	132.500	140.000	134.000
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a. Grouping Variable: z group

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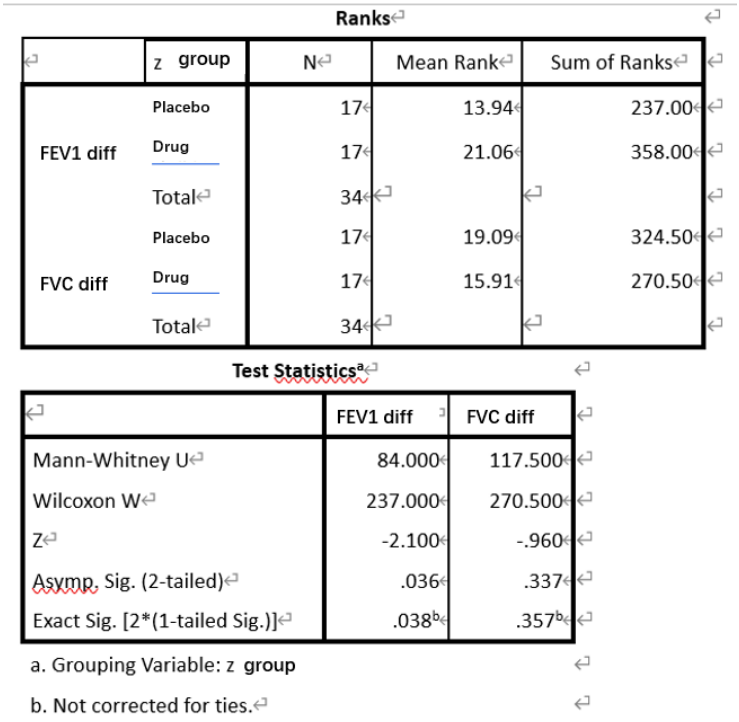
3. However, after analyzing the changes of the three pulmonary function indexes separately, it was found that the change of FEV1 in the intervention group, that is, the YQGB group, was greater than that in the COPD group, and the difference was statistically significant (P=0.036) (see Table for the rank sum test results of FEV1 difference and FVC difference). The change of FEV1/FVC% in YQGB group was greater than that in COPD group, and the difference was statistically significant (P=0.037) (see the results of FEV1/FVC% difference t test in Table). It was shown that YQGB is more effective than COPD, and it was more helpful for pulmonary function improvement in COPD patients.



Ranks

	z group	N	Mean Rank	Sum of Ranks
FEV1 diff	Placebo	17	13.94	237.00
	Drug	17	21.06	358.00
	Total	34		
FVC diff	Placebo	17	19.09	324.50
	Drug	17	15.91	270.50
	Total	34		

Test Statistics[a]

	FEV1 diff	FVC diff
Mann-Whitney U	84.000	117.500
Wilcoxon W	237.000	270.500
Z	-2.100	-.960
Asymp. Sig. (2-tailed)	.036	.337
Exact Sig. [2*(1-tailed Sig.)]	.038[b]	.357[b]

a. Grouping Variable: z group

b. Not corrected for ties.

Ranks

	Z group	N	Mean Rank	Sum of Ranks
fev1 diff	COPD	17	13.94	237.00
	COPD+YQGB	17	21.06	358.00
	Total	34		
FVC diff	COPD	17	19.09	324.50
	COPD+YQGB	17	15.91	270.50
	Total	34		

Test Statistics[a]

	fev1 diff	FVC diff
Mann-Whitney U	84.000	117.500
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Asymp. Sig. (2-tailed)	.036	.337
Exact Sig. [2*(1-tailed Sig.)]	.038[b]	.357[b]

a. Grouping Variable: z group

b. Not corrected for ties.

Changes in the text: we have modified our text as advised (see Table 2, see Page 12, line 245-249)

d-FEV1 (L) decreases from 1.4 to 1.2 following YQGB treatment, but in the meantime, FEV1 (%pred) increases from 68.7 to 77.8. It does not seem possible.

Reply: Thank you for finding the mistake, we checked the original data and re-performed the statistics.

Changes in the text: we have modified our text as advised (see Table 2)

Table 2. Pulmonary functions in the YQGB group after 24 weeks treatment.

	COPD+YQGB (n=17)			COPD (n=17)		
	Pre-treatment	Post-treatment	*t*/*Z*/p	Pre-treatment	Post-treatment	*t*/*Z*/p
FEV_1(L)	1.26(0.94,2.12)	1.28(1.02,2.16)	-2.641/0.008	1.31(0.78,1.93)	1.32(0.79,1.95)	-2.972/0.003
FVC(L)	2.11(2.03,3.42)	2.12(2.06,3.43)	-2.153/0.031	2.37(1.76,2.90)	2.37 (1.77,2.90)	-3.106/0.002
FEV_1/FVC%	55.97±9.16	57.61±10.01	2.696/0.016	55.63±11.05	55.83±11.20	0.831/0.418

e-In general, the statistics used for this figure do not seem appropriate. I might be wrong, but it seems that the authors establish their conclusion based on a t-test comparing YQGB post-treatment to PB post-treatment. Instead, the authors should compare the variation (post-treatment – pre-treatment) of each value between YQGB treatment and PB treatment. In this case, FEV1 %pred could be the right parameter to evaluate possible improvements of lung function following YQGB treatment.

Reply: Thank you for the comment. FEV1%pred is the expected value calculated according to the patient's height and weight. In COPD stable condition, the disease is stable without acute aggravation. YQGB is to strengthen the spleen and dry dampness, toning qi and strengthening the surface, the patient's appetite will improve after taking it, the weight will increase, the mental state will be improved, fatigue, shortness of breath, asthma symptoms will also be improved. It is speculated that the pulmonary function was in good condition on the day of re-examination, and the predicted value changed.

Changes in the text: No change had been made.

-Page 7, lane 255: “SIRT5 was significantly downregulated in patients with COPD compared with control patients (Figure 1A). When patients were treated with YQGB, SIRT5 mRNA levels were restored.” This is true, but there is no significant difference between the COPD and COPD+YQGB groups. Therefore, YQGB does not restore SIRT5 expression in COPD patients.

Reply: Thank you for the comment. According to the experimental results, the statistical graph was re-made, and it was found that YQGB increased the expression of SITR5 in peripheral blood of COPD patients.

Changes in the text: we updated Figure.

-Page 7, lane 257: “This condition was also confirmed by the Western blot results (Figure 1B).” Same comment as above.

Reply: Thank you for the comment. According to the experimental results, the statistical graph was re-made.

Changes in the text: we updated Figure1.

-Page 7, lane 259: “Flow cytometry revealed that mitochondrial membrane potential (JC-1) was significantly decreased in patients with COPD compared with control patients and was increased after YQGB treatment (Figure 1C).” This figure is not clear. Which window should we look at? How many subjects were used in this experiment? A conclusion cannot be drawn without quantifying the results and without statistical analysis. Please, provide quantification and stats.

Reply: Thank you for the comment. 34 patients with COPD and 17 healthy control patients, All COPD patients were randomly divided into oral YQGB group (n = 17) and placebo group (n = 17).

Changes in the text: we updated Figure1 and modified Page 13, Line 268.

-Page 7, lane 262: “The examination of mitochondrial ROS (mtROS) revealed that it was significantly elevated in patients with COPD compared with the control patients and decreased after YQGB treatment (Figure 1D).” Same as above. How many subjects were used in this experiment? A conclusion cannot be drawn without quantifying the results and without statistical analysis. Please, provide quantification and stats.

Reply: Thank you for the comment. 34 patients with COPD and 17 healthy control patients, All COPD patients were randomly divided into oral YQGB group (n = 17) and placebo group (n = 17).

Changes in the text: we updated Figure1 and modified Page 14, Line 270.

-Page 7, lane 266: “it was observed that CSE stimulation affects cell proliferation (Figure 2A) and may have toxic effects on cells. Since the result was not obvious to the naked eye, which might be caused by being reflected in the inhibition of cell organelles and other fine points. Therefore, inhibition rate was used for qualification, which refers to the inhibition ability of drugs/reagents on cell proliferation and is mainly manifested in the proliferation ability of cells and the damage to cells.” This paragraph is unclear, but since no conclusion could be drawn from this experiment, I suggest that Figure 2A be removed from the manuscript. The following MTT test is sufficient to draw conclusions regarding the proliferation rate of the cells.

Reply: Thank you for the comment. Due to shooting problems, the display of Figure 2A is not very obvious, but the cell morphology under the microscope is wrinkled and rounded, and the density is reduced. Therefore, we believe that keeping Figure 2A in the paper can support the conclusion in many aspects, and express the process and authenticity of the experiment.

Changes in the text: No change was made.

-Page 7, lane 278: "The siRNA sequences with the best knockdown rate were selected using the qRT-PCR (Figure S1)." Was siRNA 120 used in the siRNA experiments? Please, indicate which siRNA was used.

Reply: Thank you for the comment. The screening results using RT-qPCR showed that: Compared with the normal control group of HBE cells, the NC control group had no effect on the expression level of SIRT5 after transfection of siRNA. After transfection of siRNA with different numbers, the expression level of SIRT5 in all groups was decreased at different degrees, among which the knockdown effect of siRNA with number 120 was the best, so the SiRNA with this number was selected for knockdown. See table below.

Statistical table of optimal knockdown target sequence data for siRNA screening

Table 3 Statistical table of optimal knockdown target sequence data for siRNA screening

Group	N	Relative expression of Sirt5	T1	P1	T2	P2
HBE	3	1.001±0.064				
HBE+siRNA NC	3	1.057±0.023	4.617	0.32		
HBE+siRNA 119	3	0.824±0.083*	0.077	0.01	2.155	0.00
HBE+siRNA 120	3	0.483±0.074**	0.029	0.00	2.568	0.00
HBE+siRNA 141	3	0.758±0.064**	0.024	0.00	2.060	0.00

Note: T1 and P1 indicated the significance when compared with HBE group, * : $0.01 < P < 0.05$; ** : $P < 0.01$; T2 and P2 indicated the significance when compared with HBE+siRNA NC group, * : $0.01 < P < 0.05$; ** : $P < 0.01$

Changes in the text: we added Table 3 and modified Page 14, Line 286.

-Page 7, lane 283: Replace "considerably" with "significantly".

Reply: Thank you for your comments. "considerably" has been replaced with "significantly".

Changes in the text: we added Table 3 and modified Page 14, Line 289.

-Page 7, lane 285: "In addition, cell proliferation in each group was similarly observed by microscopy (Figure 3B). It suggested that YQGB treatment considerably alleviated the decreased cell proliferation ability caused by CSE stimulation." If the authors want to draw a conclusion from Figure 3B, they need to quantify the proliferation observed in these images and do a statistic analysis. Otherwise, they need to use the MTT assay used in Figure 2B (with quantification and stats).

Reply: Thank you for the comment. We are very sorry that we did not consider it thoroughly at that time. Only microscope photos were taken in this part, and CCKB and other tests were not carried out, so it was unable to be quantified.

Changes in the text: No change was made.

-Page 7, lane 299: "ATP content was significantly higher than before." This sentence does not make sense here, please remove.

Reply: Thank you for your comments. The sentence has been removed.

Changes in the text: we added Table 3 and modified Page 14, Line 299.

-Page 7, lane 299: "After the CSE group received YQGB treatment, the ATP level was alleviated." This sentence is not clear. I might be wrong but I would replace it with "After the CSE group with SIRT5 knock down received YQGB treatment, the ATP level was restored (Figure 4C)."

Reply: Thank you for helping revise the translation error.

Changes in the text: we modified Page 15, Line 308-309.

-Page 8, lane 304: "These results suggest that SIRT5 may contribute to mitochondrial dysfunction inhibited by YQGB treatment to ameliorate CSE." This sentence is not clear, can you rephrase it?

Reply: Thank you for the comment. We had rephrased the sentence.

Changes in the text: we modified Page 16, Line 313-320.

Discussion

-Page 9, lane 349: "which was consistent with previous studies" Please, add the references of the "previous studies".

Reply: Thank you for the comment. The following references had been added.

[41] Ou T, Yang W, Li W, et al. SIRT5 deficiency enhances the proliferative and therapeutic capacities of adipose-derived mesenchymal stem cells via metabolic switching. Clin Transl Med. 2020. 10(5):e172.

[42] Guedouari H, Daigle T, Scorrano L, Hebert-Chatelain E. Sirtuin 5 protects mitochondria from fragmentation and degradation during starvation. Biochim Biophys Acta Mol Cell Res. 2017. 1864(1):169-176.

[43] Liu L, Peritore C, Ginsberg J, Shih J, Arun S, Donmez G. Protective role of SIRT5 against motor deficit and dopaminergic degeneration in MPTP-induced mice model of Parkinson's disease. Behav Brain Res. 2015. 281:215-21.

[44] Li W, Yang Y, Li Y, Zhao Y, Jiang H. Sirt5 Attenuates Cisplatin-Induced Acute Kidney Injury through Regulation of Nrf2/HO-1 and Bcl-2. Biomed Res Int. 2019. 2019:4745132.

Changes in the text: we modified Page 16, Line 313-320.

Conclusion

-Page 9, lane 363: "to promoting mitochondrial dysfunction via SIRT5 inhibition." This sentence is not clear and is confusing. Can you modify it?

Reply: Thank you for the comment. The sentence had been modified.

Changes in the text: we modified Page 19, Line 404-408.

Figure legends

-Figure 1B: SIRT5 expression is detected by Western blot and not by qRT-PCR.

Reply: Thank you for the comment. The sentence had been modified.

Changes in the text: we modified Page 30, Line 622.

-Figure 1: "Ctrl: control, COPD: chronic obstructive pulmonary disease, YQGB: Yiqigubiao pill." Do not repeat it 3 times.

Reply: Thank you for the comment. The repeated sentences have been modified.

Changes in the text: we modified Page 30, Line 626.

Reviewer B

This is an interesting article about the effects of the therapeutic preparation named 'Yiqigubiao' (YQGB), which is used in traditional Chinese medicine, on COPD. The authors analyze the effects of this therapy on COPD patients and epithelial cell cultures. In the first case, its effects on lung function, blood expression of sirtuin 5 (SIRT5) and the amount of ROS, as well as on the mitochondrial membrane potential. In the case of human bronchial epithelial cells (HBE), they analyzed the effects of exposure to tobacco smoke (CSE) on the expression of SIRT5, ATP and ROS production, and also on mitochondrial membrane potential, either during free expression of SIRT5 or knocking out this deacetylase. Their results are interesting since they indicate that the use of YQGB is capable of improving patients' lung function, while increasing their expression of SIRT5, reducing the presence of ROS and improving the mitochondrial membrane potential. In HBE cultures in turn, the deleterious effects of tobacco smoke on cell proliferation, SIRT5, ATP and ROS levels, as well as on the mitochondrial membrane potential (more pronounced when SIRT5 knockout is performed) become partially reversed by the use of the above-mentioned therapy. The Discussion and conclusions are roughly appropriate to the results. However, I have some considerations and comments.

Major:

Introduction and Methodology: The vast majority of readers are unaware of the formulation of YQGB. This cannot remain as a 'closed box'. Details of composition should be included, allowing understanding on its components, preparation and their already well-known or unknown pharmacological actions. On the other hand, it would be important to explain the reason for the treatment duration, which was set at 24 weeks.

Reply: Thank you for the comment. We added the relevant information to the discussion section of the manuscript.

Changes in the text: we modified Page 18, Line 378-391.

Methods: The authors used an immortalized epithelial cell line. A pity, since a primary culture of the patients' epithelium would probably have been more appropriate, since the acute 'in vitro' exposure to tobacco smoke probably does not reflect the effects of the chronic 'in vivo' exposure suffered by real COPD patients.

Reply: Thank you for the comment. First, COPD does not require surgical treatment, so the authors could not legally obtain lung tissue. Second, YQGB is a drug for the treatment of COPD remission. Although bronchoscopy may be performed in the acute exacerbation of COPD, the acute exacerbation is not an indication for the treatment of Yiqi Gubiao pills, which does not meet the experimental enrollment conditions. Basically, patients with pulmonary lobectomy are patients with tumors, which cannot truthfully reflect the level of SIRT5 in COPD. Therefore, it is difficult to select primary cells for culture in this study. The pathological changes of COPD are mainly due to long-term chronic inflammatory reactions, which are manifested as degeneration and necrosis of airway epithelial cells, metaplasia of squamous epithelial cells and shortening of fibers in the later stage. Bronchial epithelial cells are widely used in studies related to COPD, therefore, 16HBE cells were selected for this study.

Changes in the text: No changes had been made.

Methods: In the 'Diagnosis criteria for COPD' section, the authors include sentences that are not actually ‘Methodology’ but rather considerations on COPD that may be better placed in other sections of the article.

Reply: Thank you for the comment. We had revised the diagnosis criteria.

Changes in the text: we modified Page 6, Line 108-112.

Results: the ‘improvement’ of the lung function has been based on the statistics carried out with the results obtained in each group after the administration of the drug or the placebo. However, the correct way is to analyze the differences between pre- and post- treatment between both groups of patients. Probably the simplest way would be to compare and present changes occurring in each one of these groups in absolute or percentual terms. My rough calculations indicate that, for example, FEV1 changed by 13% in those patients treated with YQGB compared to the -5% observed in those treated with placebo, a difference that is probably significant and reinforces the authors' hypothesis.

Reply: Thank you for the comment.

The two groups were compared before and after treatment as suggested. The results showed that the three pulmonary function indexes in YQGB group were significantly different before and after treatment, P<0.05; There were significant differences in FEV1 and FVC before and after treatment in COPD group (P<0.05). There was no significant difference in FEV1/FVC% before and after treatment, P>0.05; See Table 2 for details.

There was no statistical difference in the baseline data before and after treatment between YQGB and COPD groups, and the indicators in YQGB group after treatment were not higher than those in COPD group, which may be related to the slightly higher pulmonary function indicators in COPD group than YQGB group in baseline data (see table).

Test Statistics[a]

	FEV1 pt	FEV1 t	FVC pt	FVC t	FEV1/FVC pt	FEV1/FVC t
Mann-Whitney U	132.000	127.500	132.000	132.500	140.000	134.000
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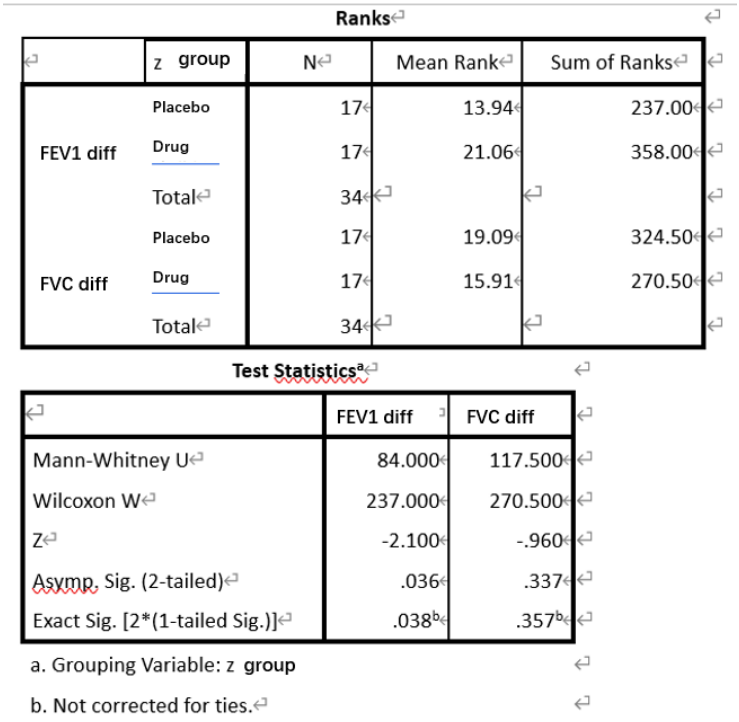
pt: before treatment
t: after treatment
a. Grouping Variable: z group
b. Not corrected for ties

3. However, after analyzing the changes of the three pulmonary function indexes separately, it was found that the change of FEV1 in the intervention group, that is, the YQGB group, was greater than that in the ~~PB~~ COPD group, and the difference was statistically significant (P=0.036) (see Table for the rank sum test results of FEV1 difference and FVC difference). The change of FEV1/FVC% in YQGB group was greater than that in ~~PB~~ COPD group, and the difference was statistically significant (P=0.037) (see the results of FEV1/FVC% difference t test in Table). It was shown that YQGB is more effective than COPD, and it was more helpful for pulmonary function improvement in COPD patients.



Ranks

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Mann-Whitney U	132.000	127.500	132.000	132.500	140.000	134.000
Wilcoxon W	285.000	280.500	285.000	285.500	293.000	287.000
Z	-.431	-.586	-.431	-.413	-.155	-.362
Asymp. Sig. (2-tailed)	.667	.558	.667	.679	.877	.718
Exact Sig. [2*(1-tailed Sig.)]	.683[b]	.563[b]	.683[b]	.683[b]	.892[b]	.734[b]

a. Grouping Variable: z group

b. Not corrected for ties.

Ranks

	Z group	N	Mean Rank	Sum of Ranks
fev1 diff	COPD	17	13.94	237.00
	COPD+YQGB	17	21.06	358.00
	Total	34		
FVC 差值	COPD	17	19.09	324.50
	COPD+YQGB	17	15.91	270.50

Total	34		

Test Statistics[a]

	fev1 diff	FVC diff
Mann-Whitney U	84.000	117.500
Wilcoxon W	237.000	270.500
Z	-2.100	-.960
Asymp. Sig. (2-tailed)	.036	.337
Exact Sig. [2*(1-tailed Sig.)]	.038[b]	.357[b]

a. Grouping Variable: z group

b. Not corrected for ties.

Changes in the text: we have modified our text as advised (see Table 2, see Page 12, line 245-249)

Minor:

Methods: 'healthy control' are NOT 'patients' (lines 147 and 280) by definition. Moreover, it is indicated that these healthy controls were ‘recruited from the outpatient clinics’ of the participating hospitals. Does the latter indicate that they were subjects with relevant comorbidities? (their elevated exposure to tobacco smoke, 44 pack/yr, seems to suggest this possibility)

Reply: Thank you for the comment. The healthy control group recruited for this study was screened from the outpatient physical examination center. According to the exclusion criteria, people who did not meet the requirements of the trial were excluded, and there were no related complications. The healthy control group included occasional smokers as well as passive smokers in the same environment, such as a partner who smoked, a co-worker who smoked at work, or chronically exposed to air pollution, and 44 packs of tobacco per year were calculated based on the number of people who smoked. As the number of cigarettes smoked is misleading, this item has been deleted and data in Table 1 has been recalculated.

Changes in the text: we have modified our text as advised (see Table 1).

Methods: Statistic analysis employed in the present study always used parametric tests. Does this indicate that all variables had a normal distribution?

Reply: Thank you for the comment.

1. Comparison of baseline data between COPD patients and healthy controls

Statistical analysis: Age and BMI were measurement data, and the results of normality test showed that their distribution obeyed normality, so they were statistically described using mean ± standard deviation (mean ± SD), and the differences between groups were statistically described using T-test. Counting data such as gender and smoking history were described using

the number of cases (n) and composition ratio (%), and Chi-square test was used to analyze the composition differences between groups. The test level α is set to 0.05.

2. Comparison of pulmonary function in patients with COPD after different treatments

Statistical analysis: The experimental type of pulmonary function change before and after treatment in COPD patients was paired design, and the data type was measurement data. The normal test was conducted on the pulmonary function change (difference) before and after treatment, and the results showed that the difference of FEV1/FVC% before and after treatment followed normal distribution, so mean ± SD was used for statistical description. Paired T-test was used for differences before and after treatment. The difference between FEV1 and FVC before and after treatment did not follow normal distribution, so the median and interquartile spacing were used for statistical description (median, P25-P75), and the paired rank sum test was used for statistical analysis. Test level α=0.05.

Tests of Normality

	Z group	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
FEV1/FVC diff	Placebo	.147	17	.200*	.958	17	.590
	Drug	.153	17	.200*	.936	17	.276
FEV1 diff	Placebo	.227	17	.020	.878	17	.029
	Drug	.123	17	.200*	.954	17	.527
FVC diff	Placebo	.363	17	.000	.712	17	.000
	Drug	.239	17	.011	.793	17	.002

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Tests of Normality

	Z group	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
FEV1FVC diff	COPD	.147	17	.200*	.958	17	.590
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fev1 diff	COPD	.227	17	.020	.878	17	.029
	COPD+YQGB	.123	17	.200*	.954	17	.527
FVC diff	COPD	.363	17	.000	.712	17	.000
	COPD+YQGB	.239	17	.011	.793	17	.002

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Changes in the text: we modified Table 1 and Table 2.

Results, table 1: The very high exposure to tobacco of COPD patients is at least surprising (121 pack/year on average). The same accounts for 'healthy controls (44 pack/year). This deserves at least some comment in the Discussion.

Reply: Thank you for the comment. We modified the whole first graph in discussion section.

Changes in the text: we modified Page 16, Line 317-336.

Results, table 2: The number 1 in FEV1 and FEV1/FVC must be in subscript. On the other hand, the units for FEV1 and relative FVC are not '%' but '% pred.'; and the units for FEV1/FVC are percentages (i.e. '%'). This should be improved.

Reply: Thank you for the comment. We modified table 2.

Changes in the text: we have modified our text as advised (see Table 2)

Results, Graph 1: It would also be interesting to indicate with asterisks the significances between COPD and COPD+YQGB situations. The same accounts for the inclusion of a bar with the results of the COPD+placebo group.

Reply: figure 1 legend has been revised.

Changes in the text: Figure 1. YQGB treatment causes changes in mitochondria.

The mRNA levels of SIRT5 in the mitochondria of the control group, COPD group, and YQGB-treated group were detected by qRT-PCR. * $P < 0.05$, COPD vs Ctrl. Ctrl: control, COPD: chronic obstructive pulmonary disease, YQGB: Yiqigubiao pill.

The expression of SIRT5 in the mitochondria of the control group, COPD group, and YQGB-treated group was detected by Western blot. Ctrl: control, COPD: chronic obstructive pulmonary disease, YQGB: Yiqigubiao pill.

The abundance of mitochondrial JC-1 was detected by flow cytometry. * $P < 0.05$, COPD vs Ctrl. Ctrl: control, COPD: chronic obstructive pulmonary disease, YQGB: Yiqigubiao pill.

D. The levels of mitochondrial reactive oxygen species were detected by flow cytometry. Ctrl: control, COPD: chronic obstructive pulmonary disease, YQGB: Yiqigubiao pill.

Results, Graph 2: It should be indicated to which comparison the asterisks correspond (are they representing the comparisons of each one of the situations with '0'?)

Reply: figure 2 legend has been revised.

Changes in the text: Figure 2. Cell proliferation was inhibited after the cigarette smoke extract (CSE) stimulation of the human bronchial epithelial (HBE) cells. A. Microscopic observation at different times after the HBE cells were stimulated with different concentrations of CSE. Scale bar, 50 μm. B. Inhibitory rate of the CSE stimulation on HBE cell proliferation. * $0.01 < P < 0.05$ vs. HBE group, ** $P < 0.01$ vs. HBE group.

Discussion, first paragraph: The first paragraph should emphasize the most relevant findings of the study, and not be a general discussion on COPD.

Reply: Thank you for the comment. We modified the whole first graph in discussion section.

Changes in the text: we modified Page 16, Line 317-336.

Discussion, first paragraph: Early changes in the FEF25-75 with tobacco smoking should not be a topic to be mentioned since this variable presents a wide variability for the same individual.

Reply: Thank you for the comment. We modified the whole first graph in discussion section.

Changes in the text: we modified Page 16, Line 317-336.

Conclusion: It should state 'reversing mitochondrial dysfunction' and not 'promoting mitochondrial dysfunction'. Likewise, this action would be through the 'restoration of the expression of SIRT5' and not its 'inhibition', as the current wording states.

Reply: Thank you for the comment. We modified the conclusion section.

Changes in the text: we modified Page 20, Line 411-416.