

# ARSIP EMAIL DARI TORMJ

**MPS**  
Powered by Eureka

Edy Parwanto  
User Portal

- Dashboard
- New Submissions
- Email Notifications
- Support
- User Manuals

Welcome to your Dashboard Author / Dashboard

### ASSOCIATE AUTHOR SUBMISSIONS

Journal	Reference No.	Date	Title	Status
No Submissions				

### NEWLY SUBMITTED ARTICLES

Show 5 articles Search

Journal	Reference No.	Date	Title	Status
TORMJ	BMS-TORMJ-2023-18	2023-10-29	Low doses of inhaled cigarette smoke altered rat lung histomorphologic and gene expression of the p53 gene	Ready to Publish

Showing 1 to 1 of 1 entries Previous 1 Next

### AWAITING REVISION











Journal	Reference No.	Date	Title	Status
---------	---------------	------	-------	--------

## EMAIL NOTIFICATIONS

Search By Reference...

Submit

Reset

Journal	Subject	Email Sent On	Message
TORMJ	Acceptance Letter   BMS-TORMJ-2023-19	08-May-2024	 <a href="#">view</a>
TORMJ	Thankyou For Uploading Proof Corrections   BMS-TORMJ-2023-19	02-May-2024	 <a href="#">view</a>
TORMJ	COVERING LETTER 2ND Galley Proofs   BMS-TORMJ-2023-19	26-Apr-2024	 <a href="#">view</a>
TORMJ	Thankyou For Uploading Proof Corrections   BMS-TORMJ-2023-19	01-Apr-2024	 <a href="#">view</a>
TORMJ	COVERING LETTER 1ST Galley Proofs   BMS-TORMJ-2023-19	01-Apr-2024	 <a href="#">view</a>
TORMJ	In verification inquiry [BMS-TORMJ-2023-19]	15-Mar-2024	 <a href="#">view</a>
TORMJ	Publication Fee   BMS-TORMJ-2023-19   BSP-19173	13-Mar-2024	 <a href="#">view</a>
TORMJ	Preliminary Acceptance  BMS-TORMJ-2023-19	13-Mar-2024	 <a href="#">view</a>
TORMJ	Thank you for completing the Revision   BMS-TORMJ-2023-19	26-Feb-2024	 <a href="#">view</a>
TORMJ	TORMJ Manuscript Revision Required   BMS-TORMJ-2023-19	22-Feb-2024	 <a href="#">view</a>

Total Rows: 15

1

2 (<https://bentham.manuscriptpoint.com/messages/Dashboard/GetAuthorEmails/2>)

> (<https://bentham.manuscriptpoint.com/messages/Dashboard/GetAuthorEmails/2>)

---

## Email Message:

Reference#: BMS-TORMJ-2023-19

Submission Title: Low doses of kretek cigarette smoke altered rat lung histometric, and overexpression of the p53 gene

Dear Dr. Edy Parwanto,

Thank you for your submission to "The Open Respiratory Medicine Journal". It will be sent to the Editor-in-Chief for his initial provisional approval. Once this is obtained it will be passed on to our Author Support Services department (BASS), for an initial assessment of the manuscript. BASS is a specialized department that helps authors and editors to make sure that the manuscript becomes ready for submission into the peer-review process. The manuscript is being processed on the clear understanding that it contains original work that has neither been published earlier nor has it been simultaneously been submitted for publications elsewhere. In case this is not so, then kindly let us know immediately.

Please note that Bentham Open uses **Cross Check's iThenticate** software to check for similarities between the submitted and already published material to minimize any chances of plagiarism.

Further, as per Bentham Open's Ethical Guidelines for Publication, all manuscripts are processed with the understanding that all authors and co-authors have reviewed and accordingly approved the manuscript before final submission to avoid any conflicts of interest later. Our ethical policies can be viewed on the Journal's website.

**Articles which are well referenced (100 or more references) may have high chances of acceptance by referees and they are likely to attract a greater number of citations The recommended number of references as per norm (<https://clarivate.com/essays/impact-factor/>) for Review Articles is approximately 100 or more, for Research Articles 75 or more, for Mini Reviews 75 or more & for Letter Articles 50 or more.**

In case of any doubt or conflict please contact us.

Your manuscript has been assigned to the following Editor/Manager to whom all correspondence may kindly be addressed:

tormj@benthamopen.net

### Few tips to enhance the visibility & citations of your article:

- If you don't already have an ORCID ID please create one at <https://orcid.org/register>
- **Selection of Appropriate Keywords:** Please choose important and relevant keywords that researchers in your field could be searching for so that your paper can appear in many database searches. In biomedical fields, MeSH terms are a good 'common vocabulary' source to draw keywords from: see <https://www.nlm.nih.gov/mesh/meshhome.html>
- The names of the authors should be provided according to the previous citations (or as the authors would want them to be published) along with their institutional affiliations, current addresses, telephone, cell & fax numbers and the email address. Email address must be provided with an asterisk in front of the name of the principal author. The corresponding author(s) should be designated and their complete address, business telephone and fax numbers and e-mail address must be stated to receive correspondence and galley proof. It is also suggested to regularly update your profile in SCOPUS and other databases.
- Authors should avoid the use of non-standard abbreviations and question marks in titles.

**For articles submitted from non-English speaking countries, authors should have their articles carefully checked and the language corrected by a native English speaker. Alternatively, authors may want to avail Bentham's English language correction service. For a quote please contact Bentham editorial office.** Please note that your article will not be processed till you have completed and returned the attached fee form by replying to

this e-mail.

Looking forward to a successful collaboration in the publication of your article.

Sincerely,

Editorial Office  
The Open Respiratory Medicine Journal  
Bentham Open  
tormj@benthamopen.net  
<https://www.benthamopen.com/TORMJ/home/>

Bentham Open is constantly striving to improve its publication practices. If you are not satisfied with any procedure of the processing of your manuscript, then please let us know at the following email address with full details:

Note: For Assistance please contact: [info@benthamopen.net](mailto:info@benthamopen.net)

For complaints please contact: [complaint@benthamopen.net](mailto:complaint@benthamopen.net)

Please click below to Download Fee Form and Copyright Form.  
[copyright\\_form.pdf](#)

Close

TORMJ	TORMJ Quick Track Notification   BMS-TORMJ-2023-19	04-Oct-2023	view
-------	--	-------------	------

## Email Message

Dear Dr. Edy Parwanto,

Thank you very much for submitting your manuscript entitled "Low doses of kretek cigarette smoke altered rat lung histometric, and overexpression of the p53 gene" for consideration for publication in the "The Open Respiratory Medicine Journal".

You may wish to avail our new fast publication services called QUICK TRACK, which allows urgent publication of those papers that the authors feel require rapid publication.

QUICK TRACK allows online publication within 2 weeks of receipt of the final approved galley proofs from the authors. The total publication time, from the date of first receipt of manuscript to its online publication is 6 weeks subject to its acceptance by the referees and modification (if any) by the authors within one week.

Authors who have availed Quick Track service in a Bentham Open journal will be entitled for an exclusive 30% discount if they again wish to avail the same services in any Bentham Open journal within the next 12 months.

Authors are encouraged to submit the revised manuscript within 48 hours for timely publication of their manuscript submitted in QUICK TRACK category.

If you would like to avail the QUICK TRACK services, please complete the attached Quick Track Publication Form

and send it back by e-mail at [quicktrack@benthamopen.net](mailto:quicktrack@benthamopen.net) . We must receive this form within the next 4 working days so that we can proceed with the peer reviewing process.

You will be charged US \$300 processing fee (non-refundable) and an additional \$450 publication fee (refundable if paper is not accepted for publication). The quick track publication fee together with the Bentham Open publication charges, as described in the attached Timelines and Payment Schedule will be payable before online publication of the paper. If the paper is rejected, there will be no further charges other than the \$300 already paid.

Please note that whether you opt for the QUICK TRACK facility or not, standard reviewing practices will be followed, which will not in any way affect the acceptance or rejection of the manuscript by the reviewers.

Thank you in advance for your consideration. We look forward to hearing from you soon.

Kind regards,

Editorial Office  
The Open Respiratory Medicine Journal  
Bentham Open

If you are interested to avail Quick Track service, Please [Click Here](#).

Note: For complaints contact: [complaint@benthamopen.net](mailto:complaint@benthamopen.net)

Close

TORMJ

Manuscript Initial Submission letter [BMS-TORMJ-2023-19]

07-Oct-2023

## Email Message

**Reference#: BMS-TORMJ-2023-19**

Submission Title: Low doses of kretek cigarette smoke altered rat lung histometric, and overexpression of the p53 gene

Dear Dr. Edy Parwanto,

We are pleased to inform you that your article entitled, "**Low doses of kretek cigarette smoke altered rat lung histometric, and overexpression of the p53 gene**" has been initially approved by the Editorial Advisory Board of the journal "**The Open Respiratory Medicine Journal**". to proceed further.

This is to inform you that currently your article is under editorial evaluation by Bentham Author Services Support (BASS) editors.

The Bentham Authors Services Support (BASS) editors consist of competent professionals who perform detailed, initial scrutiny of all submitted manuscripts. to assist authors in fine-tuning their manuscripts. This will help reduce the errors that may delay the review and publication process later. The core aim of the BASS editors is to ensure the complete, correct, and concise incorporation of all details needed at the initial stage, to ensure smooth and timely processing of the article to the peer-review stage.

Your article will be evaluated by the BASS editors for the following things:

- \* Language editing
- \* Figure quality checking/enhancement
- \* Reference duplication-checking/formatting
- \* Quality Control (Plagiarism/ Similarity Checking)

- \* Ethical protocols and overall text organization
- \* Citation of References / Figures / Tables etc.
- \* Selection of right category/nature of the article
- \* Funding details/acknowledgement etc.

Please note that on successful completion of this initial review, your article will then be subjected to a rigorous and neutral peer-review process, before an acceptance decision can be taken.

Thanks and Regards,

Editorial Office  
-----

Close

TORMJ	Thank you for completing the Revision   BMS-TORMJ-2023-19	29-Oct-2023
-------	---	-------------

## Email Message

Submission Title: Low doses of kretek cigarette smoke altered rat lung histometric, and overexpression of the p53 gene.

Dear Dr. Parwanto,

Thank you very much for submitting your revised manuscript, BMS-TORMJ-2023-19. We hope to successfully collaborate with you in the future as well. Please do let us know if you face any issues.

Regards,

Editorial Office  
The Open Respiratory Medicine Journal  
Bentham Open

TORMJ	Successful Completion from Initial Scrutiny   BMS-TORMJ-2023-19	14-Nov-2023
-------	---	-------------

## Email Message

**Reference#: BMS-TORMJ-2023-19**

Submission Title: Low doses of kretek cigarette smoke altered rat lung histometric, and overexpression of the p53 gene

Dear Dr. parwanto,

This is to update you about your manuscript titled "Low doses of kretek cigarette smoke altered rat lung histometric, and overexpression of the p53 gene " submitted to the journal "The Open Respiratory Medicine Journal". Your manuscript has been passed through Initial scrutiny successfully. Currently the manuscript is at the peer review stage.

You may track all the stages of publication online until your manuscript is finalized and is ready for publication. Log onto JMS <https://bentham.manuscriptpoint.com> and click the article reference number from the available list of your submitted manuscripts to view the detailed status at every stage of the peer-review process and editorial decision.

Best Regards

Editorial Office

TORMJ	TORMJ Manuscript Revision Required   BMS-TORMJ-2023-19	22-Feb-2024
-------	--	-------------

## Email Message

Reference#: BMS-TORMJ-2023-19

Submission Title: Low doses of kretek cigarette smoke altered rat lung histometric, and overexpression of the p53 gene

Dear Dr. edy parwanto,

Thank you for submitting to "The Open Respiratory Medicine Journal". Your manuscript has been reviewed, and it needs revision (comments given below/ attached). You may download the correct manuscript version by [Clicking here](#). You are encouraged to carefully revise the manuscript, highlighting the exact changes made.

**Our publication policy requires the return of your revised manuscript within 5 days from the date of receipt of this message.**

When submitting a revised manuscript for further consideration, please ensure that all the suggested changes are incorporated into the manuscript. Also, highlight or mark up the changes made throughout the manuscript for ease of reference.

**Please note that if the revised version is received within 5 days, it will be completed and forwarded for electronic publication (subject to acceptance by the reviewers and journal editors) in the next 10 days from the date of receipt of the revised accepted manuscript. Please make sure all payment formalities are finished before publication.**

The final decision will be taken after the nature of your response letter and revision.

**Authors from non-English speaking countries should ensure to have their articles corrected by a native English speaker for any grammatical, stylistic and typographical errors.**

Authors who are native English speakers should ensure that their article has been carefully checked for language, grammar, and style (where appropriate). This is in your interest as it will substantially reduce the time taken for the publication of your article.

**Note:** Please make sure to submit your revised manuscript through our Journal Management System <https://bentham.manuscriptpoint.com/> otherwise it may face delays in processing.

Response letter must be based on the following pattern:

Reviewer 1/problem 1:

Reply 1:

Reviewer 2/problem 2:

Reply 2:

**Reviewer Comments:**

**REVIEWER 1/Problem 1**

**Comments:** It is a valuable study. We need this kind of study against to industrial try to sell the tobacco product, getting them as if they are non-harmful. The manuscript clearly show the negative harmful effects on lungs, and p53 which is genetical factor for lung cancer. The study will be beneficial for readers and open the new research. I am positive for publication. My English is not my main language, may be good if it check for language by someone native speaker.

**REVIEWER 2/Problem 2**

**Comments:** It is a interesting article, although the information is not new. The difference here is that it talks about an specific kind of cigarette. I have some questions: 1- The discussion talks about changes in the color of the filters, although this was not mention in the methods and /or the results. 2- Are kretek cigarettes different to those used in America, for example? This should be explained, as it is vital to the originality of the manuscript- 3- Do you know if the tobacco plant used in these cigarettes is contaminated with cadmium? There are several reports that state that tobacco plants and contaminated, therefore all cigarettes contain this heavy metal, which is responsible for many of the alterations seen in this study. See Watanabe et al, 1987 (Cadmium and lead contents of cigarettes produced in various areas of the world); Elinder et al, 1983 (Cadmium exposure from smoking cigarettes: Variations with time and country where purchased). 4- Although p53 is increased, this just shows some kind of protection, not really suggesting there is cancer or cell propagation. Did you measure some apoptosis markers for instance? English is very poor, therefore the whole manuscript needs to be read (and corrected) by a English native speaker. I will just point some errors but there are more . ABSTRACT METHODS Line 1- delete OF RESULTS. Line 4. Delete THEY Introduction This phrase is too colloquial. Try to rephrase. "The other reports demonstrated that smoking is a risk factor for various types of diseases, because it is better for smokers to stop smoking before the age of 40 years". Paragraph 2. Line 2. There are some words that need separation (spaces). Paragraph 3 needs rewriting. It is missing a verb. Paragraph 4. This sentence needs rewriting. "People who smoke, gases, and particulate matter in the smoke pouring from the mouth to the alveoli." paragraph 5. this sentence needs rewriting. "In addition, it has been demonstrated about the lower respiratory system of mice (bronchioles, alveolar passages, and alveoli) both qualitatively and quantitatively [18]". What was demonstrated? Also, rewrite the sentence "A recent study also reported that rats were given conventional cigarette smoke, collagen deposition appearance in the bronchioles, as well as intrabronchiolar mucus" Paragraph 6. Rewrite the sentence "Quantitative changes in the bronchioles and respiratory bronchioles are important so that the diagnosis related to lung tissue disorders determined more objectively." The word bronhcoles, has a typo Ethical clearance and...??? And what? There is a verb missing. WAS obtained. Animal care The whole paragraph should be rewritten. There are several verbs missing. Treatment of Sprague .... Some verbs are missing It is EXPOSED TO not exposed BY

**REVIEWER 3/Problem 3**

**Comments:** There are several questions which are raised by this report. First, what is new? Animal models of tobacco smoking have long shown changes consistent with human COPD. Second, what do you mean by "low dose"? The continuous exposure was sufficient to affect the animals' hair. What method did you use to conclude that the animal exposure was consistent with low dose ordinary human exposure? Third were the lungs prepared under standard conditions. It is usual that when fixing lungs for morphometric analysis they be inflated to a standard volume. Fourth why was simple pathology not done. The data could be consistent with constriction of the small bronchioles but there also could have been interstitial fibrosis. Finally, are the P53 data new? Are they consistent with other studies showing gene disruption in models like these.



Sincerely,

Ambreen Irshad  
Senior Editor  
Bentham Open  
ambreenirshad@benthamopen.net

Bentham Science is constantly striving to improve its publication practices. If you are not satisfied with any procedure of the processing of your manuscript, then please let us know at the following email address with full details:

For complaints please contact: [complaint@benthamopen.net](mailto:complaint@benthamopen.net)

**Attachments:**

[Close](#)

TORMJ	Thank you for completing the Revision   BMS-TORMJ-2023-19	26-Feb-2024
-------	---	-------------

## Email Message

Submission Title: Low doses of kretek cigarette smoke altered rat lung histometric, and overexpression of the p53 gene

.

Dear Dr. Parwanto,

Thank you very much for submitting your revised manuscript, BMS-TORMJ-2023-19. We hope to successfully collaborate with you in the future as well. Please do let us know if you face any issues.

Regards,

Editorial Office  
The Open Respiratory Medicine Journal  
Bentham Open

TORMJ	Preliminary Acceptance   BMS-TORMJ-2023-19	13-Mar-2024
-------	--	-------------

## Email Message

**Reference#:** BMS-TORMJ-2023-19

**Submission Title:** Low doses of kretek cigarette smoke altered rat lung histometric, and overexpression of the p53 gene

Dear Dr. Edy Parwanto,

On behalf of Bentham Science, I am pleased to inform you that your article titled "**Low doses of kretek cigarette smoke altered rat lung histometric, and overexpression of the p53 gene**" with Reference No. "BMS-TORMJ-2023-19" has been found generally acceptable for publication in the journal "**The Open Respiratory Medicine Journal**" journal.

You will now need to pay the processing fee for Research Article only, either via the online payment link in the invoice sent to you or, via a bank transfer (evidence of which would need to be sent to us), before your article is published.

Certain aspects of your submission relating to the quality of language, plagiarism/ similarity, figures and use of AI tools (if any) may require additional scrutiny by our Quality Assurance Department before final acceptance and publication.

Attached is your digitally signed copyright letter for your records.

We appreciate your valuable contribution to "**The Open Respiratory Medicine Journal**" and look forward to successful collaboration in the future.

With warm regards,

Editorial Office  
The Open Respiratory Medicine Journal  
Bentham Open

For complaints contact: [complaint@benthamscience.net](mailto:complaint@benthamscience.net)  
Contribute to our [Newsletter](#): Send us updates about your research to [newsletter@benthamscience.net](mailto:newsletter@benthamscience.net).  
[Sign up](#) to receive updates from Bentham Science.

Close

TORMJ	Publication Fee   BMS-TORMJ-2023-19   BSP-19173	13-Mar-2024
-------	---	-------------

## Email Message

Dear edy parwanto,

Please find below the invoice for your manuscript named as 'Low doses of kretek cigarette smoke altered rat lung histometric, and overexpression of the p53 gene ' .

Kindly remit the payment at the earliest.

You may pay below invoice by [Clicking Here](#)

We thank you in advance, for your kind co-operation in the matter.

If you have any queries please do not hesitate to contact us.

Sincerely,

Leena Menon  
BENTHAM SCIENCE PUBLISHERS  
EXE.SUITE Y #2  
SAIF ZONE, SHARJAH  
UAE  
TEL: 009716 5571132  
FAX: 009716 5571134  
leenamenon@benthamscience.net

---

**BENTHAM SCIENCE PUBLISHERS LTD.  
(FZE)**

---

Invoice No. BSP-19173  
Invoice Type Bentham Invoice  
Date: 13-03-2024

BENTHAM SCIENCE PUBLISHERS LTD. (FZE)  
Executive Suite Y 2, P.O. Box 7917  
SAIF Zone, Sharjah  
United Arab Emirates 100385232200003  
Tel: +9716 5571132 Fax: +9716 5571134  
Email: leenamenon@benthamscience.net

---

**The Open  
Respiratory  
Medicine  
Journal**

---

Invoice to  
**edy parwanto**  
Trisakti University, biology,  
Indonesia

Ship to  
**edy parwanto**  
Trisakti University, biology, Indonesia

Qty	Description	Your Order / Reference	Unit Price	Amount US\$
-----	-------------	------------------------	------------	-------------

1	<b>Ref#:</b> BMS-TORMJ-2023-19 <b>Article Title:</b> Low doses of kretek cigarette smoke altered rat lung histometric, and overexpression of the p53 gene <b>Journal :</b> The Open Respiratory Medicine Journal	Article Processing Charges	1005	1005 <b>Discount: 0%</b>
			Total Amount:	1005
			VAT Charges:	0
<b>Open Payment:</b>			<b>1005</b>	<b>TOTAL US\$: 1005</b>

\* If you wish to pay with VISA, MASTERCARD or AMEX, please click on "Pay with Paypal" button below:.



**For payment by Bank Transfer**  
**Beneficiary Name:** Bentham Science Publishers Ltd (FZE)  
**SWIFT:** NBFUAEAFDXB  
**Bank Name & Address:** National Bank of Fujairah PJSC, Bank Street Dubai, P.O. Box 2979, Dubai, United Arab Emirates  
**Account No:** 012001225454  
**IBAN ( International Bank Account Number):** AE520380000012001225454  
**Note:** For wire transfers, provision of our IBAN number is a must. Please also ensure that reference is made to our invoice number (BSP-19173), to avoid your payment not being traced. Remitter and Receiver bank charges have to be borne by the Remitter.  
Please email us the bank SWIFT remittance copy once the transfer is made.

\* Please note that we do not accept cheque payments for amount less than **USD 500**.

Close

TORMJ	In verification inquiry [BMS-TORMJ-2023-19]	15-Mar-2024
-------	---	-------------

## Email Message

Dear Dr. edy parwanto,

With reference to your article entitled "**Low doses of kretek cigarette smoke altered rat lung histometric, and overexpression of the p53 gene**" submitted in the journal (**The Open Respiratory Medicine Journal TORMJ**).

While checking your article it has been observed that animals were the part of your study that's why kindly provide us the attached filled ARRIVE checklist and also provide the sample size and purchasing details of the animal used in your article.

Editorial Office  
The Open Respiratory Medicine Journal  
Bentham Open

TORMJ	COVERING LETTER 1ST Galley Proofs   BMS-TORMJ-2023-19	01-Apr-2024
-------	---	-------------

## Email Message

**URGENT**

Dear Dr. Parwanto,

The composed version of your article is available on the dashboard of the Online System. I shall be grateful if you could kindly carefully check the composed version for any potential errors, missing lines/paragraphs, and errors in figures/diagrams, etc.

Moreover, as a part of the publication process, the manuscript has undergone copyediting for minor grammatical inconsistencies and specifically for some significant ambiguities (where necessary) to bring clarity to the content. You are therefore requested to read the proof thoroughly and send approval for all the corrections made in order to ensure that the original meaning of the content has not been altered in case of significant changes in the text.

**Answer all inquiries.** The production team's inquiries are listed here. Please ensure you respond to all queries since you cannot submit the corrections until they are all answered.

**Q. 1: Reveal the source of data and materials mentioned in the manuscript, in support of the findings which will be presented in the following format in the article.**

“The data supporting the findings of the article is available in the [Mention repository name] at [Mention URL], reference number [Mention reference number]”.

- Authors may deposit their datasets openly to Zenodo Repository, in addition to their own or their institutional archives. For more details please visit <https://openpublichealthjournal.com/availability-of-data-materials.php>

**Q. 2: Kindly provide the information if the study received any funds/grants. Funding details must include:**

- Name of funding agency.
- Funder ID.
- Awards/Grant number.

**Q. 3: Could you please provide the sources for each figure and tables? (Published/reproduced material should not be included unless you have obtained written permission from the copyright holder. You have not mentioned the sources in the figures and tables.)**

**Q.4. Please complete the highlighted references in the reference section and provide them in the journal’s specific format.**

**Kindly return the corrected proofs and replies to the queries of the manuscript within 48 hours. Upon receipt of your reply, the manuscript will be finalized for publication within a week.**

**Please make sure all payment formalities are finished before publication.**

Kindly acknowledge the receipt of this e-mail.

With best wishes,  
Ms. Ambreen irshad  
Editorial Manager  
E-mail: [ambreen@benthamopen.net](mailto:ambreen@benthamopen.net)  
<https://www.linkedin.com/company/benthamopen>  
[galleyproofs@benthamopen.net](mailto:galleyproofs@benthamopen.net)

Close

TORMJ	Thankyou For Uploading Proof Corrections   BMS-TORMJ-2023-19	01-Apr-2024
-------	--	-------------

## Email Message

Submission Title : Low doses of kretek cigarette smoke altered rat lung histometric, and overexpression of the p53 gene

Dear Dr. Parwanto

Thank you very much for submitting proof corrections of your manuscript BMS-TORMJ-2023-19.

We hope to successfully collaborate with you in the future as well. Please do let us know if you face any issues.

Regards  
Editorial Office  
The Open Respiratory Medicine Journal  
Bentham Open

TORMJ	COVERING LETTER 2ND Galley Proofs   BMS-TORMJ-2023-19	26-Apr-2024
-------	---	-------------

## Email Message

### **REF. FINAL PROOFS**

Dear Dr. Parwanto,

With reference to your email regarding the proofs corrections. The revised and composed version of your paper is attached for your reference. Could you please carefully check the composed version for any potential errors and suggest any corrections that need to be incorporated before its publication.

**In case we do not receive your response within 8 hours, we will be compelled to proceed with the manuscript in its present form and no further corrections shall be incorporated.**

-----

Looking forward to a prompt response in this regard.

With best wishes,

**Sobia Adnan**

Proofreading Department

Close

TORMJ

Thankyou For Uploading Proof Corrections | BMS-TORMJ-2023-19

02-May-2024

## Email Message

Submission Title : Low doses of kretek cigarette smoke altered rat lung histometric, and overexpression of the p53 gene

Dear Dr. Parwanto

Thank you very much for submitting proof corrections of your manuscript BMS-TORMJ-2023-19.

We hope to successfully collaborate with you in the future as well. Please do let us know if you face any issues.

Regards  
Editorial Office  
The Open Respiratory Medicine Journal  
Bentham Open



## Email Message

Dear Dr. Edy Parwanto,

I am pleased to inform you that your article titled " Low doses of kretek cigarette smoke altered rat lung histometric, and overexpression of the p53 gene

" ", with Reference No. BMS-TORMJ-2023-19," has been accepted for publication in the journal " The Open Respiratory Medicine Journal " following an independent peer review.

In the next few days, the article will be published on the website and various indexing agencies will index it.

We appreciate your valuable contribution to " The Open Respiratory Medicine Journal " and look forward to a successful collaboration in the future.

With warm regards,

Editorial Office  
The Open Respiratory Medicine Journal  
Bentham Open

---

## Figure Improvement Query | BMS-TORMJ-2023-19

1 message

---

**Bentham Graphics Dept.** <support@benthamartwork.com>  
Reply-To: "Bentham Graphics Dept." <artwork@benthamscience.net>  
To: Parwanto <edyparwanto@trisakti.ac.id>  
Cc: artwork@benthamscience.net

Mon, Oct 16, 2023 at 6:23 PM

**Monday, October 16, 2023**

**Ref #:** 128539

**MPS Reference Number #** @mpsrefno

Dear Dr. Parwanto,

This is with reference to your article entitled "Low doses of kretek cigarette smoke altered rat lung histometric, and overexpression of the p53 gene", submitted for possible publication in the journal "The Open Respiratory Medicine Journal". During graphics assessment, it has been observed that the figure(s) no. **9**, embedded in your article, have not been provided according to the recommended parameters [**please see attached technical details**]. For your reference, we have attached sample images, both before and after figure improvement.

It is, therefore, requested to have your figures improved.

If you cannot arrange to do this yourself, you may also consider availing our professional Figure Improvement services, at a special rates of US\$135 for up to five figures, with any additional figures being charged at US \$20 each, by **clicking below**:

### Figure Improvement Request

or copy paste below URL in your browser:

<http://ims.itspk.com/admin/menuscrypt/cart.php?param=MTI4pNTMt5>


The total cost for improvement of your figure(s) no. 9, in the submitted manuscript, will be US \$135.

**Please note that no article will be published with substandard figures.**

**Please also note, that improved figures, alone, do not guarantee the final publication of your article. The final acceptance/rejection decision on the manuscript will be taken by the EIC, based on the quality of the article and independent peer review.**

Regards,  
Graphic Department,

---

 **128539\_lab\_report.pdf**  
687K

## Receipt for Your Payment to EurekaScience

1 pesan

service@intl.paypal.com <service@intl.paypal.com>  
Kepada: Edy Parwanto <edy.parwanto@gmail.com>

16 Oktober 2023 pukul 20.14

Dear Edy Parwanto,



# You paid \$135,00 \$ USD to EurekaScience

[View or Manage Payment](#)

**Transaction ID**

4T6863990Y3060302

**Transaction date**

16 October 2023

**Merchant**

EurekaScience

**Shipping address**

Edy Parwanto  
jl garuda 5, d 6 nomor 10, papan mas,  
mangun jaya, tambun selatan, bekasi,  
jawa barat, Indonesia  
bekasi WEST JAVA 17510  
Indonesia

<b>Subtotal</b>	\$135,00 \$ USD
<b>Total</b>	\$135,00 \$ USD
<b>Payment</b>	\$135,00 \$ USD

Charge will appear on your credit card statement as "PAYPAL \*EUREKASCIEN"

---

**Paid EurekaScience with**

Visa-3795

\$135,00 \$ USD

[View or Manage Payment](#)

**Issues with this transaction?**

You have 180 days from the date of the transaction to open a dispute in the Resolution Center.



---

[Help & Contact](#) | [Security](#) | [Apps](#)



PayPal is committed to preventing fraudulent emails. Emails from PayPal will always contain your full name. [Learn to identify phishing](#)

Please don't reply to this email. To get in touch with us, click [Help & Contact](#).

Not sure why you received this email? [Learn more](#)

Copyright © 1999-2023 PayPal. All rights reserved.

PayPal Pte. Ltd. is licensed by the Monetary Authority of Singapore as a Major Payment Institution under the Payment Services Act 2019.

PayPal RT001736:en\_US(en-ID):1.4.0:f529230fb3c3e

# JAGA KEAMANAN DATA KARTU KREDIT ANDA

Jangan Mudah Menyebarkan Data Kartu Kredit  
Kepada Siapapun Termasuk ke Pihak Bank

Kode OTP

Nomor  
Kartu Kredit

Masa Berlaku  
Kartu Kredit

3 Nomor CVC/CVV  
di Belakang Kartu



## ENJOY OUR SPECIAL OFFERS

SHOW BOARDING PASS  
GET BEVERAGES  
FOR **IDR 1**

DISCOUNT  
**15%**

With BNI Credit Card & BNI Debit Emerald until October 31<sup>st</sup>, 2023



STARBUCKS®



More Info



1500046  
BNI Call

APPLY NOW  
bit.ly/applykartukreditbni

BNI  
Rewards Point

## Lembar Penagihan Kartu Kredit BNI

<p><b>Yth. Bapak/Ibu</b> <b>EDY PARWANTO</b> <b>UNIVERSITAS TRISAKTI</b> <b>BAG FAK KEDOKTERAN</b></p> <p><b>JL KYAI TAPA GROGOL</b> <b>JAKARTA BARAT</b> <b>11440</b> 7371069C1710-000187</p>	<p><b>Tanggal Cetak</b> 17-10-2023</p> <p><b>VISA</b> </p> <p style="text-align: right;">Halaman 01 dari 01</p>
--	--

Tanggal Transaksi	Tanggal Pembukuan	Rincian Transaksi Anda	Jumlah (Rp)	Informasi Kredit
4105-05XX-XXXX-3795		TAGIHAN BULAN LALU EDY PARWANTO	17.500	BATAS KREDIT 10.000.000 BATAS PENARIKAN TUNAI 5.000.000
16-10-2023	17-10-2023	PAYPAL *EUREKASCIEN 4029357733 SG 135.00 (USD1=IDR16306,65)	2.201.398	SISA KREDIT 7.781.100 SISA PENARIKAN TUNAI 5.000.000
17-10-2023	17-10-2023	IURAN TAHUNAN	300.000	
17-10-2023	17-10-2023	PEMBEBASAN IURAN TAHUN KEDUA	300.000 CR	
		TOTAL TAGIHAN BULAN INI	2.218.898	
				<b>Ringkasan Belanja dan Pembayaran</b>
				TAGIHAN BULAN LALU 17.500 PEMBAYARAN 0 PEMBELANJAAN 2.201.398 PENARIKAN TUNAI 0 BIAYA ADM & BUNGA 300.000 TAGIHAN BULAN INI 2.218.898 PEMBAYARAN MINIMUM 111.000 TANGGAL JATUH TEMPO 06-11-2023 KOLEKTIBILITAS KREDIT 1
				<b>Informasi BNI Rewards Point</b>
				JUMLAH POIN BULAN LALU : 8.264 JUMLAH POIN BULAN INI : 880 JUMLAH POIN DITUKARKAN : 0 (-) ----- - JUMLAH POIN TERSEDIA : 9.144 POIN KADALUARSA 17/09/2024 : 8.264
				JAGA KERAHASIAAN DATA KARTU KREDIT DAN DATA TRANSAKSI ANDA. DARI PIHAK MANAPUN TERMASUK PIHAK BNI

### Info dan Promo Bulan Ini

<p><b>1. Informasi Pengkinian Data:</b> Segera perbaharui data Anda (seperti alamat rumah, alamat kantor dan nomor handphone) jika mengalami perubahan. Info selengkapnya cek <a href="https://bit.ly/bni-pengkiniandata">bit.ly/bni-pengkiniandata</a> atau melalui BNI Call 1500046.</p> <p><b>2. Informasi Asuransi Perisai Plus:</b> Informasi mengenai perubahan manfaat asuransi Perisai Plus. info selengkapnya cek <a href="http://bit.ly/BNI-PP">http://bit.ly/BNI-PP</a></p>	<p><b>Informasi Kartu Kredit BNI:</b></p> <p><b>1. Informasi perpanjangan terkait Biaya Keterlambatan Pembayaran dan Batas Minimum Pembayaran Kartu Kredit.</b> Info selengkapnya klik <a href="https://bit.ly/bnicinfo1">bit.ly/bnicinfo1</a></p> <p><b>2. Kenalan Yuk Dengan Fitur Contactless Kartu Kredit BNI.</b> Info selengkapnya klik <a href="https://bit.ly/bnictless">bit.ly/bnictless</a></p> <p><b>3. Informasi Penyesuaian Biaya Pembayaran Kartu Kredit BNI Melalui Menu Transfer.</b> Info selengkapnya klik <a href="https://bit.ly/paymentfeeKKBNI">bit.ly/paymentfeeKKBNI</a></p>	<p><b>1. Informasi perubahan biaya konversi cicilan Kartu Kredit BNI efektif per 20 Oktober 2023.</b> Info selengkapnya klik <a href="https://bit.ly/inst-biayakonversi">https://bit.ly/inst-biayakonversi</a></p> <p><b>2. Info promo Kartu Kredit BNI</b> klik <a href="https://bniexperience.bni.co.id">https://bniexperience.bni.co.id</a></p>
--	--	--

# Informasi untuk Anda

## Tanggal Cetak Tagihan, Tagihan Bulan Ini, Tanggal Jatuh Tempo dan Minimum Pembayaran

Tanggal Cetak Tagihan adalah tanggal terakhir transaksi dan pembayaran dibukukan ke lembar tagihan Pemegang Kartu untuk bulan berjalan. Apabila tanggal cetak tagihan bertepatan dengan hari libur nasional (termasuk hari Sabtu dan Minggu), maka tanggal terakhir untuk pembukuan transaksi dan pembayaran bergeser ke hari kerja sebelumnya. Tagihan bulan ini adalah tagihan baru yang harus Anda bayar sebelum atau pada tanggal jatuh tempo. Tanggal jatuh tempo adalah tanggal terakhir pembayaran Anda diterima oleh BNI. Apabila tanggal jatuh tempo bertepatan dengan hari libur nasional, maka tanggal jatuh tempo bergeser ke hari kerja berikutnya. Minimum pembayaran Anda setiap bulan adalah 5% dari total tagihan atau minimum Rp 100.000,- (Khusus Kartu Kredit BNI Jenis Silver dan Emas minimum Rp 50.000,-).

### Cara Pembayaran

Nama Bank	Jenis Channel Pembayaran	Biaya*
BNI	ATM, SMS Banking dan Internet Banking	tanpa biaya
	Kantor Cabang	Rp 25.000,-
	Phone Banking	Rp 3.000,-
	Autodebet	tanpa biaya

Mitra Bank	Biaya*
Tokopedia E-Commerce	Rp 9.000,-
Bukalapak E-Commerce	Rp 9.000,-

Pembayaran melalui Kliring/RTGS dan Menu Transfer	Biaya*
Transfer pembayaran melalui Bank lain (Kliring/RTGS)	Rp 4.000,-
Menu Transfer Bank (Jaringan LINK, ATM Bersama, Alto dan Prima)/e-Wallet	Rp 7.500,-

\* Biaya di atas dapat berubah sewaktu-waktu dan akan disampaikan melalui pemberitahuan tertulis atau media lainnya.

- Transfer pembayaran dapat juga dilakukan melalui bank lain (kliring/RTGS) dengan mencantumkan nomor Kartu Kredit BNI (16 digit) diikuti dengan nama Pemegang Kartu (besar biaya ditetapkan oleh masing-masing bank).
- Untuk menghindari denda keterlambatan, silakan lakukan pembayaran 2 s/d 3 hari kerja sebelum tanggal jatuh tempo tagihan.
- Pembayaran melalui Bank lain akan efektif diterima BNI 2 s/d 3 hari kerja setelah pembayaran dilakukan.

Bunga dan Biaya Administrasi*	Keterangan
Bunga pembelanjaan	1,75%/bulan
Bunga penarikan uang tunai	1,75%/bulan
Biaya pengambilan uang tunai	6% dari jumlah penarikan uang tunai atau minimal Rp 100.000,- (Signature & World) 6% dari jumlah penarikan uang tunai atau minimal Rp 125.000,- (Titanium, Platinum & Precious) 6% dari jumlah penarikan uang tunai atau minimal Rp 150.000,- (Silver & Gold)
Biaya keterlambatan pembayaran	1% dari total tagihan atau maksimal Rp 100.000,-
Biaya pelampauan batas kredit	Rp 150.000,-
Biaya fasilitas Dana Tunai	4% atau minimal Rp 30.000,-
Biaya penggantian kartu	Rp 100.000,- (Kecuali Infinite)
Biaya permintaan salinan slip	Rp 30.000,-/transaksi (Dalam Negeri), Rp 100.000,-/transaksi (Luar Negeri)
Biaya cetak tagihan	Rp 20.000,-/tagihan
Biaya cetak ulang tagihan	Rp 5.000,- /tagihan (via fax) Rp 30.000,-/tagihan (via mailing) Free untuk peserta e billing (via email)


\* Bunga dan biaya di atas dapat berubah sewaktu-waktu dan akan disampaikan melalui pemberitahuan tertulis atau media lainnya. Informasi selengkapnya dapat dilihat melalui link berikut <https://bit.ly/bni-pay>

### Tagihan yang Tidak Sesuai

Apabila terdapat tagihan yang tidak sesuai pada lembar penagihan Anda, hubungi Layanan 24 Jam BNI Call selambat-lambatnya 30 hari sejak tanggal penagihan Anda. Bila tidak ada pelaporan pada periode tersebut, kami menganggap transaksi Anda telah sesuai.

### Kartu Hilang

Apabila Kartu Kredit BNI Anda hilang atau tercuri, segera hubungi Layanan 24 Jam BNI. Transaksi yang terjadi sebelum Anda menghubungi BNI Call, akan menjadi tanggung jawab Pemegang Kartu.

Gunting di sini 

Jika data Anda mengalami perubahan, mohon isi formulir ini dan fax ke BNI Call di (021) 2554 1203 :

Nama : \_\_\_\_\_  
No. Kartu Kredit BNI : \_\_\_\_\_  
Alamat Baru : \_\_\_\_\_  
RT/RW: \_\_\_\_\_ Kota: \_\_\_\_\_ Kode Pos   
Nomor Telepon (K) \_\_\_\_\_ (R) \_\_\_\_\_ (HP) \_\_\_\_\_  
E-mail : \_\_\_\_\_  
Tanda Tangan : \_\_\_\_\_

### Untuk Informasi :

#### Hubungi Layanan 24 Jam BNI Call

Telepon : 1500046  
Fax : (021) 2554 1203  
E-mail : [bnicall@bni.co.id](mailto:bnicall@bni.co.id)  
Website : [www.bni.co.id](http://www.bni.co.id)  
Pos : PO. BOX 8888/ JKTM Jakarta 12700

**1500046**  
BNI Call

# Eureka Science FZE

Eureka Science FZE  
Executive Suite Y, #3, P.O. Box 121223  
SAIF Zone, Sharjah  
United Arab Emirates  
Tel: +971 6 5575783 Fax: +971 6 5575784  
Email: [services@eurekascience.net](mailto:services@eurekascience.net)

Invoice No.	6873
Date:	2023/10/16
Status:	<b>Payment Confirmed</b>

Figure

Invoice to

<b>Edy Parwanto</b>
---------------------

Ship to

<b>Edy Parwanto</b>
---------------------

Qty	Description	Your Order / Reference	Unit Price	Amount US\$
1	Improved Figures			135
Total Amount:				135
<b>TOTAL Amount Paid US\$:</b>				<b>135</b>

**In case of any query, kindly email at: [azhar@benthamsience.net](mailto:azhar@benthamsience.net)**



---

## TORMJ Manuscript Revision Required | BMS-TORMJ-2023-19

---

The Open Respiratory Medicine Journal <admin@bentham.manuscriptpoint.com>

Thu, Feb 22, 2024 at  
11:34 AM

Reply-To: The Open Respiratory Medicine Journal <meg@benthamscience.net>

To: edyparwanto@trisakti.ac.id

Cc: maryam@benthamopen.net

Reference#: BMS-TORMJ-2023-19

Submission Title: Low doses of kretek cigarette smoke altered rat lung histometric, and overexpression of the p53 gene

Dear Dr. edy parwanto,

Thank you for submitting to "The Open Respiratory Medicine Journal". Your manuscript has been reviewed, and it needs revision (comments given below/ attached). You may download the correct manuscript version by [Clicking here](#) . You are encouraged to carefully revise the manuscript, highlighting the exact changes made.

**Our publication policy requires the return of your revised manuscript within 5 days from the date of receipt of this message.**

When submitting a revised manuscript for further consideration, please ensure that all the suggested changes are incorporated into the manuscript. Also, highlight or mark up the changes made throughout the manuscript for ease of reference.

**Please note that if the revised version is received **within 5 days**, it will be completed and forwarded for electronic publication (subject to acceptance by the reviewers and journal editors) **in the next 10 days from the date of receipt of the revised accepted manuscript**. Please make sure all payment formalities are finished before publication.**

The final decision will be taken after the nature of your response letter and revision.

**Authors from non-English speaking countries should ensure to have their articles corrected by a native English speaker for any grammatical, stylistic and typographical errors.**

Authors who are native English speakers should ensure that their article has been carefully checked for language, grammar, and style (where appropriate). This is in your interest as it will substantially reduce the time taken for the publication of your article.

**Note:** Please make sure to submit your revised manuscript through our Journal Management System <https://bentham.manuscriptpoint.com/> otherwise it may face delays in processing.

Response letter must be based on the following pattern:

Reviewer 1/problem 1:

Reply 1:

Reviewer 2/problem 2:

Reply 2:

**Reviewer Comments:**

**REVIEWER 1/Problem 1**

**Comments:** It is the valuable study. We need this kind of study against to industrial try to sell the tobacco product, getting them as if they are non-harmful. The manuscript clearly show the negative harmful effects on lungs, and p53 which is genetical factor for lung cancer. The study will be beneficial for readers and open the new research. I am positive for publication. My English is not my main language, may be good if it check for language by someone native speaker.

**REVIEWER 2/Problem 2**

**Comments:** It is a interesting article, although the information is not new. The difference here is that it talks about an specific kind of cigarette. I have some questions: 1- The discussion talks about changes in the color of the filters, although

this was not mention in the methods and /or the results. 2- Are kretek cigarettes different to those used in America, for example? This should be explained, as it is vital to the originality of the manuscript- 3- Do you know if the tobacco plant used in these cigarettes is contaminated with cadmium? There are several reports that state that tobacco plants and contaminated, therefore all cigarettes contain this heavy metal, which is responsible for many of the alterations seen in this study. See Watanabe et al, 1987 (Cadmium and lead contents of cigarettes produced in various areas of the world); Elinder et al, 1983 (Cadmium exposure from smoking cigarettes: Variations with time and country where purchased). 4- Although p53 is increased, this just shows some kind of protection, not really suggesting there is cancer or cell propagation. Did you measure some apoptosis markers for instance? English is very poor, therefore the whole manuscript needs to be read (and corrected) by a English native speaker. I will just point some errors but there are more .

ABSTRACT METHODS Line 1- delete OF RESULTS. Line 4. Delete THEY Introduction This phrase is too colloquial. Try to rephrase. "The other reports demonstrated that smoking is a risk factor for various types of diseases, because it is better for smokers to stop smoking before the age of 40 years". Paragraph 2. Line 2. There are some words that need separation (spaces). Paragraph 3 needs rewriting. It is missing a verb. Paragraph 4. This sentence needs rewriting. "People who smoke, gases, and particulate matter in the smoke pouring from the mouth to the alveoli." paragraph 5. this sentence needs rewriting. "In addition, it has been demonstrated about the lower respiratory system of mice (bronchioles, alveolar passages, and alveoli) both qualitatively and quantitatively [18]". What was demonstrated? Also, rewrite the sentence "A recent study also reported that rats were given conventional cigarette smoke, collagen deposition appearance in the bronchioles, as well as intrabronchiolar mucus" Paragraph 6. Rewrite the sentence "Quantitative changes in the bronchioles and respiratory bronchioles are important so that the diagnosis related to lung tissue disorders determined more objectively." The word bronhcioles, has a typo Ethical clearance and...??? And what? There is a verb missing. WAS obtained. Animal care The whole paragraph should be rewritten. There are several verbs missing. Treatment of Sprague .... Some verbs are missing It is EXPOSED TO not exposed BY

### REVIEWER 3/Problem 3

**Comments:** There are several questions which are raised by this report. First, what is new? Animal models of tobacco smoking have long shown changes consistent with human COPD. Second, what do you mean by "low dose"? The continuous exposure was sufficient to affect the animals' hair. What method did you use to conclude that the animal exposure was consistent with low dose ordinary human exposure? Third were the lungs prepared under standard conditions. It is usual that when fixing lungs for morphometric analysis they be inflated to a standard volume. Fourth why was simple pathology not done. The data could be consistent with constriction of the small bronchioles but there also could have been interstitial fibrosis. Finally, are the P53 data new? Are they consistent with other studies showing gene disruption in models like these.

Sincerely,

Ambreen Irshad  
Senior Editor  
Bentham Open  
[ambreenirshad@benthamopen.net](mailto:ambreenirshad@benthamopen.net)

Bentham Science is constantly striving to improve its publication practices. If you are not satisfied with any procedure of the processing of your manuscript, then please let us know at the following email address with full details:

For complaints please contact: [complaint@benthamopen.net](mailto:complaint@benthamopen.net)

### Attachments:

To unsubscribe from MPS and stop receiving emails further. **Please Click [Here](#)**

Powered by [Bentham Manuscript Processing System](#)

---

## Publication Fee | BMS-TORMJ-2023-19 | BSP-19173

---

**Leena Menon** <admin@bentham.manuscriptpoint.com>  
Reply-To: Leena Menon <leenamenon@benthamsience.net>  
To: edyparwanto@trisakti.ac.id

Wed, Mar 13, 2024 at 10:42 AM

Cc: leenamenon@benthamsience.net, ambreenirshad@benthamsience.net, azhar@benthamsience.net

Dear edy parwanto,

Please find below the invoice for your manuscript named as 'Low doses of kretek cigarette smoke altered rat lung histometric, and overexpression of the p53 gene ' .

Kindly remit the payment at the earliest.

You may pay below invoice by [Clicking Here](#)

We thank you in advance, for your kind co-operation in the matter.

If you have any queries please do not hesitate to contact us.

Sincerely,

Leena Menon  
BENTHAM SCIENCE PUBLISHERS  
EXE.SUITE Y #2  
SAIF ZONE, SHARJAH  
UAE  
TEL: 009716 5571132  
FAX: 009716 5571134  
[leenamenon@benthamsience.net](mailto:leenamenon@benthamsience.net)

---

## BENTHAM SCIENCE PUBLISHERS LTD. (FZE)

---

*BENTHAM SCIENCE PUBLISHERS LTD. (FZE)  
Executive Suite Y 2, P.O. Box 7917  
SAIF Zone, Sharjah  
United Arab Emirates 100385232200003  
Tel: +9716 5571132 Fax: +9716 5571134  
Email: [leenamenon@benthamsience.net](mailto:leenamenon@benthamsience.net)*

Invoice No.	BSP-19173
Invoice Type	Bentham Invoice
Date:	13-03-2024

---

**The Open  
Respiratory  
Medicine  
Journal**

---

Invoice  
to

**edy parwanto**  
Trisakti University, biology, Indonesia

Ship to

**edy parwanto**  
Trisakti University, biology, Indonesia

Qty	Description	Your Order / Reference	Unit Price	Amount US\$
1	<b>Ref#:</b> BMS-TORMJ-2023-19 <b>Article Title:</b> Low doses of kretek cigarette smoke altered rat lung histometric, and overexpression of the p53 gene <b>Journal :</b> The Open Respiratory Medicine Journal	Article Processing Charges	1005	1005 <b>Discount:</b> <b>0%</b>
			Total Amount:	1005
			VAT Charges:	0
<b>Open Payment:</b>			<b>1005</b>	<b>TOTAL US\$:</b> <b>1005</b>

\* If you wish to pay with VISA, MASTERCARD or AMEX, please click on "Pay with Paypal" button below:.



**For payment by Bank Transfer**

**Beneficiary Name:** Bentham Science Publishers Ltd (FZE)

**SWIFT:** NBFUAEAFDXB

**Bank Name & Address:** National Bank of Fujairah PJSC, Bank Street Dubai, P.O. Box 2979, Dubai, United Arab Emirates

**Account No:** 012001225454

**IBAN ( International Bank Account Number):** AE520380000012001225454

**Note:** For wire transfers, provision of our IBAN number is a must. Please also ensure that reference is made to our invoice number (BSP-19173), to avoid your payment not being traced. Remitter and Receiver bank charges have to be borne by the Remitter.

Please email us the bank SWIFT remittance copy once the transfer is made.

\* Please note that we do not accept cheque payments for amount less than **USD 500**.

To unsubscribe from MPS and stop receiving emails further. **Please Click [Here](#)**

Powered by [Bentham Manuscript Processing System](#)

## Reference Duplication Checking Report

**File Name:** BMS-TORMJ-2023-19

**Article Title:** Low doses of kretek cigarette smoke altered rat lung histometric,

**Article Type:** Research Article

**Reference System ID:** REFCHK-114541

**Reference Check Number:** RCN1047021710393887

**BMS Code:** BMS-TORMJ-2023-19

**Total References:** 55

**Total Bentham References %:** 0%

**Total Unique References:** 55

**Journal References:** 47

**Web References:** 8

**Report Date Time:** 2024-03-14 05:24:10

**Total PMID:** 34

**Total DOI:** 46

### Author Self Citations

S.NO.	Journal Name	Article Title	Author Name	Total References	Self Citations	Self Citation Percentage
REFCHK-114541	The Open Respiratory Medicine Journal	Low doses of kretek cigarette smoke altered rat lung histometric,	Edy Parwanto	55	3	5.45%
REFCHK-114541	The Open Respiratory Medicine Journal	Low doses of kretek cigarette smoke altered rat lung histometric,	Husnun Amalia	55	1	1.82%

### Self Cited Author's Detail

S.NO.	Reference Number	Reference Article Title	Author Name	Main Author
1	r55	The change of cell biometric and its nucleus on cervical-	Edy Parwanto	

S.NO.	Reference Number	Reference Article Title	Author Name	Main Author
		squamous-epithelial-cell with GA genotype of Fas-promoter-670 gene, high-risk human papillomavirus and Candida species infection: A case report. <i>Bali Med J.</i> <b>2021</b> ; 10 74-81.		
2	r38	Decreased density of pyramidal cells in the cerebral cortex, and Purkinje cells in the cerebellar cortex of Sprague-Dawley rats after being exposed to filtered kretek cigarette smoke. <i>J Bio Res.</i> <b>2023</b> ; 96 1-6.	Edy Parwanto	
3	r37	Effects of low-dose filtered kretek cigarette smoke on bronchial smooth muscle in male Sprague-Dawley rats. <i>Univ Med.</i> <b>2023</b> ; 42 259-271.	Edy Parwanto	
4	r38	Decreased density of pyramidal cells in the cerebral cortex, and Purkinje cells in the cerebellar cortex of Sprague-Dawley rats after being exposed to filtered kretek cigarette smoke. <i>J Bio Res.</i> <b>2023</b> ; 96 1-6.	Husnun Amalia	

---

## Payment Received Against Invoice Number (6873)

2 messages

---

**Billing Department** <admin@ims.itspk.com>  
To: Edy Parwanto <edyparwanto@trisakti.ac.id>

Mon, Oct 16, 2023 at 8:14 PM

Dear Dr.Edy Parwanto,

Thank you for your payment of \$135,

Payment Receipt is attached below for your reference

Best Regards,  
IMS Support Team

---

 **Payment-confirmation-Receipt-6873.pdf**  
45K

---

**Billing Department** <admin@ims.itspk.com>  
To: Edy Parwanto <edyparwanto@trisakti.ac.id>

Mon, Oct 16, 2023 at 8:14 PM

[Quoted text hidden]

---

 **Payment-confirmation-Receipt-6873.pdf**  
45K

---

## Thankyou For Uploading Proof Corrections | BMS-TORMJ-2023-19

---

**The Open Respiratory Medicine Journal** <admin@bentham.manuscriptpoint.com>

Mon, Apr 1, 2024 at 7:07 PM

Reply-To: The Open Respiratory Medicine Journal <tormj@benthamopen.net>

To: edyparwanto@trisakti.ac.id

Cc: raheela@benthamsience.net

Submission Title : Low doses of kretek cigarette smoke altered rat lung histometric,  
and overexpression of the p53 gene

Dear Dr. Parwanto

Thank you very much for submitting proof corrections of your manuscript BMS-TORMJ-2023-19.

We hope to successfully collaborate with you in the future as well. Please do let us know if you face any issues.

Regards  
Editorial Office  
The Open Respiratory Medicine Journal  
Bentham Open


To unsubscribe from MPS and stop receiving emails further. **Please Click [Here](#)**

Powered by [Bentham Manuscript Processing System](#)



# Low Doses of Kretek Cigarette Smoke Altered Rat Lung Histometric, and Overexpression of the p53 Gene



Edy Parwanto<sup>1,\*</sup> , David Tjahyadi<sup>2</sup>, Sisca Sisca<sup>1</sup>, Husnun Amalia<sup>3</sup>, Nany Hairunisa<sup>4</sup>, Hosea Jaya Edy<sup>5</sup>, Ashaolu Victoria Oladimeji<sup>6</sup> and Noureddine Djebli<sup>7</sup>

<sup>1</sup>Department of Biology, Faculty of Medicine, Universitas Trisakti, Jl. Kyai Tapa, Kampus B, No.260 Grogol 11440, Jakarta, Indonesia

<sup>2</sup>Department of Histology, Faculty of Medicine, Universitas Trisakti, Jakarta, Indonesia

<sup>3</sup>Department of Ophthalmology, Faculty of Medicine, Universitas Trisakti, Jakarta, Indonesia

<sup>4</sup>Department of Occupational Medicine, Faculty of Medicine, Universitas Trisakti, Jakarta, Indonesia

<sup>5</sup>Study Program of Pharmacy, Faculty of Math, and Natural Sciences, Universitas Sam Ratulangi, Manado, Indonesia

<sup>6</sup>Department of Chemistry, Loyola Institute of Frontier Energy, Loyola College, Chennai, India

<sup>7</sup>Department of Biologie, Faculty of Natural and Life Sciences, Abdelhamid Ibn Badis University, Mostaganem, Algeria

## Abstract:

**Background:** The components of kretek cigarettes include tobacco as the main part, clove, and sauce. Filtered kretek cigarettes are kretek cigarettes that have one end filtered. Cigarette smoke contributes to the disruption of the respiratory system, so it is necessary to know the effect of low doses of cigarette smoke on changes in the histometric of the respiratory system, and whether it affects p53 gene expression. This study aims to determine changes in the histometric of the respiratory system and p53 gene expression.

**Methods:** In this study, we used Sprague-Dawley rats. Group I of rats breathing normal air, were not exposed to filtered kretek cigarette smoke (as a control). Group II of rats, as a treatment group, were exposed to filtered kretek cigarette smoke 1 stick/day for 3 months. The results of lung histometry measurements and p53 gene expression between groups were analyzed using the Independent Sample T-test. The difference between groups is significant if the test results show  $P < 0.05$ .

**Results:** Bronchioles length, width, area, and perimeter in group I were  $40.55 \pm 1.57 \mu\text{m}$ ,  $14.82 \pm 0.41 \mu\text{m}$ ,  $494.61 \pm 5.62 \mu\text{m}^2$ , and  $233.87 \pm 4.51 \mu\text{m}$ , respectively. Bronchioles length, width, area, and perimeter in group II were  $30.76 \pm 0.78 \mu\text{m}$ ,  $9.28 \pm 0.40 \mu\text{m}$ ,  $297.32 \pm 2.53 \mu\text{m}^2$ , and  $177.84 \pm 5.15 \mu\text{m}$ , respectively. The area and perimeter of respiratory bronchioles in group I were  $17.68 \pm 0.49 \mu\text{m}^2$ , and  $26.60 \pm 0.52 \mu\text{m}$  respectively, while those in group II were  $19.28 \pm 0.35 \mu\text{m}^2$ , and  $29.28 \pm 0.35 \mu\text{m}$ , respectively. Mucus was found in the bronchioles and respiratory bronchioles in group II, however, there was no visible mucus observed in group I. In addition, it was also concluded that exposure to low doses of filtered kretek cigarette smoke, 1 cigarette/day for 3 months, increased the expression of the p53 gene in the lungs of rats.

**Conclusion:** The size of bronchioles in rats decreased after being exposed to filtered kretek cigarette smoke 1 stick/day for 3 months, while the size of respiratory bronchioles increased. In addition, exposure to cigarette smoke increased the expression of the p53 gene in the rat lungs.

**Keywords:** Filtered kretek cigarettes smoke, Histometric, Bronchioles, Respiratory bronchioles, p53 gene, Tobacco.

© 2024 The Author(s). Published by Bentham Open.

This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International Public License (CC-BY 4.0), a copy of which is available at: <https://creativecommons.org/licenses/by/4.0/legalcode>. This license permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

\*Address correspondence to this author at the Department of Biology, Faculty of Medicine, Universitas Trisakti, Jl. Kyai Tapa, Kampus B, No.260 Grogol 11440, Jakarta-Indonesia; E-mail: [edyparwanto@trisakti.ac.id](mailto:edyparwanto@trisakti.ac.id)

Cite as: Parwanto E, Tjahyadi D, Sisca S, Amalia H, Hairunisa N, Edy HJ, Oladimeji A, Djebli N. Low Doses of Kretek Cigarette Smoke Altered Rat Lung Histometric, and Overexpression of the p53 Gene. Open Respir Med J. 2024; 18: e18743064285619. <http://dx.doi.org/10.2174/0118743064285619240327055359>



Received: October 29, 2023  
Revised: February 26, 2024  
Accepted: March 13, 2024  
Published: ?? ??, 2024



Send Orders for Reprints to  
[reprints@benthamscience.net](mailto:reprints@benthamscience.net)

## 1. INTRODUCTION

In Indonesia, many people smoke, especially among young people [1]. Cigarettes can be obtained from the market, but some are made by residents, manually. The main ingredient of cigarettes is tobacco. Cigarettes are one of the major health problems in the world. Even so, many people smoke. It has been reported that cigarettes kill more than 8 million people each year in the world. More than 7 million of those deaths are the result of direct tobacco use, while around 1.2 million are the result of non-smokers being exposed to second-hand smoke [2]. The other reports demonstrated that smoking is a risk factor for various types of diseases, because smokers should stop smoking before the age of 40 years [3]. Especially Indonesia, is one of the countries in Southeast Asia whose population is mostly smokers [4]. Moreover, the number of smokers in Indonesia is very large. In addition, filtered kretek cigarettes are in great demand by most active smokers in Indonesia. This fact shows that most Indonesians smoke kretek.

There are several types of cigarettes in Indonesia, including filtered kretek cigarettes. Generally, filtered kretek cigarettes are made in the factory using machines and are equipped with a filter at one end. In an earlier period, the kretek cigarettes were not filtered at one end. Until now, on the market circulating filtered kretek cigarettes, and non-filtered kretek cigarettes. Filtered kretek cigarettes contain tobacco (as the main ingredient) that is mixed with cloves and sauces [5]. In addition to the added cloves, there are other ingredients to complement the taste of kretek cigarettes, namely sauce.

The scientific name of cultivated tobacco is *Nicotiana tabacum* Linn. Based on a previous report, the Indonesia cigarette industry ( $\pm 80\%$ ) used local tobacco such as Temanggung tobacco, Weleri tobacco, Yogyakarta tobacco, Muntilan Tobacco, Boyolali tobacco, Paiton tobacco, Kasturi tobacco, and Madura tobacco [6]. The dried clove flower buds (*Syzygium aromaticum* (L.) Merr. & L.M.Perry) [7] were used as dough in filtered kretek cigarettes. Filtered kretek cigarette sauce is a unique taste of cigarettes [8]. In Klaten district and its surroundings (Central Java Province, Indonesia), kretek cigarette sauce is known as "woor".

Cigarette smoke contains a complex mixture of chemical compounds. More than 4000 chemical compounds in cigarette smoke have been identified [9]. Cigarette smoke is known to consist of gas (92%) and solid or particulate matter (8%) [10]. Recent research demonstrates that particulate matter emissions from kretek cigarettes can be very high. This is clearly dangerous for both active smokers and non-smokers who are exposed to cigarette smoke [11]. People who smoke, gases and particulate matter in the smoke pour from the mouth to the alveoli. It has been demonstrated that there was deposition of cigarette smoke particles in various locations of the human respiratory tract [12].

Furthermore, it has been demonstrated that cigarette smoke is associated with bronchopulmonary dysplasia

[13]. In addition, cigarette smoke also has free radicals that cause airway disorders, resulting in lung abnormalities. Moreover, cigarette smoke contains particles that damage lung tissue, causing permanent damage called chronic obstructive pulmonary disease (COPD) [14, 15].

Generally, a rat's respiratory system consists of the nose, pharynx, larynx, trachea, primary bronchi, and lungs (bronchioles and alveoli) [16]. The latest research demonstrated that components of rat lung structure are blood vessels, bronchi, terminal bronchi, respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli [17]. In addition, it has been demonstrated about the lower respiratory system of mice (bronchioles, alveolar passages, and alveoli) both qualitatively and quantitatively [18]. A previous study reported that the respiratory systems of rats exposed to gas showed increased tissue elasticity and tissue resistance [19]. A recent study also reported that rats were given conventional cigarette smoke, collagen deposition appearance in the bronchioles, as well as intrabronchiolar mucus [20].

In addition, it is also shown that cigarette smoke affects the anatomical structures of the respiratory system [21]. Another study also showed that smokers have experienced basal cell hyperplasia, mucous cell hyperplasia, and squamous metaplasia [22]. In the application of COPD diagnosis, both qualitative and quantitative assessments are needed [23]. This research is important because bronchioles and respiratory bronchioles are an important part of the lungs associated with the alveoli. We intend to present the quantitative changes in bronchioles, and respiratory bronchioles caused by the effects of filtered kretek cigarette smoke. Quantitative changes in the bronchioles and respiratory bronchioles are important so that the diagnosis related to lung tissue disorders can be determined more objectively. Apart from that, it also needs to be proven whether low doses of kretek cigarette smoke affect gene expression, especially p53. We know that the p53 gene is used as a tumour marker as a key cellular process such as proliferation, apoptosis, and metabolism to suppress tumorigenesis [24]. Therefore, based on the description above, it is necessary to research the histometric of bronchioles, and respiratory bronchioles in Sprague-Dawley rats after treatment with filtered kretek cigarette smoke. Likewise, it is also necessary to prove the dangers of low-dose smoking on over-expression of the p53 gene.

## 2. MATERIALS AND METHODS

### 2.1. Preparation of Filtered Kretek Cigarettes

The type of tobacco used as an ingredient in filtered kretek cigarettes was local tobacco. The types of local tobacco (*Nicotiana tabacum* Linn.) were collected from areas in Manisrenggo District, Klaten Regency, Central Java Province, Indonesia. Cloves (*Syzygium aromaticum* Merr. & L.M. Perry) used in filtered kretek cigarette dough were dried clove flowers, collected from areas in the Logede Village, Karangnongko District, Klaten Regency, Central Java Province, Indonesia. The sauce

known as “woor,” used for filtered kretek cigarettes was a special ingredient that was always used to mix cigarettes manually. These materials were used to make filtered kretek cigarettes manually.

**2.2. Ethical Clearance**

The ethical clearance of this research was obtained from the Research Ethics Commission, Faculty of Medicine, Universitas Trisakti, Jakarta, Indonesia (No. 184/KER-FK/VIII/2018).

**2.3. Flowchart of Experimental Rat Treatment**

The flowchart of experimental rat treatment is presented in Fig. (1).

**2.4. Animal Care**

We used *Rattus norvegicus*, Sprague-Dawley strain. The number of rats per group was calculated using formula  $(n-1) (t-1) \geq 15$ . For each treatment group a minimum of 8 rats were required. Sixty male rats were used in this study. The rats included in this study were 2-3 months old and weighed 150-200 grams. Determination of rat health was carried out after examination by a Veterinary. Rats were caged (individually) in an air-

conditioned room and the room temperature was set to  $22 \pm 3$  °C, humidity  $55 \pm 5\%$ , and artificial fluorescent lamps (12:12 hour light and dark cycle). The rats fed and drank in the libitum according to the standard. There are 2 stages in this research, namely the 1<sup>st</sup> stage in the form of rat acclimatization and the 2<sup>nd</sup> stage was the treatment of giving filtered kretek cigarette smoke to rats.

**2.4.1. Purchasing Details of the Experimental Rats**

Type of animal is rat, Amount 16, Gender is Male Age is 2.5 months Weight 150-200 grams. Systematica (American Fancy Rat and Mouse Association, 2004) - Kingdom - Filum - Sub Filum - Class - Ordo - Sub Ordo - Family - Genus - Species - Strain: Animalia: Chordata: Vertebrata: Mammalia: Rodentia: Myomorpha: Muridae: Rattus: Rattus norvegicus: Sprague Dawley

**2.5. Treatment on Sprague-dawley Rats**

Trial of filtered kretek cigarette smoke on rats was carried out at The Veterinary Medical Teaching Hospital (Rumah Sakit Hewan Pendidikan=RSHP), Faculty of Veterinary Medicine of Bogor Agricultural University. This research was conducted between November 2019 - April 2020.

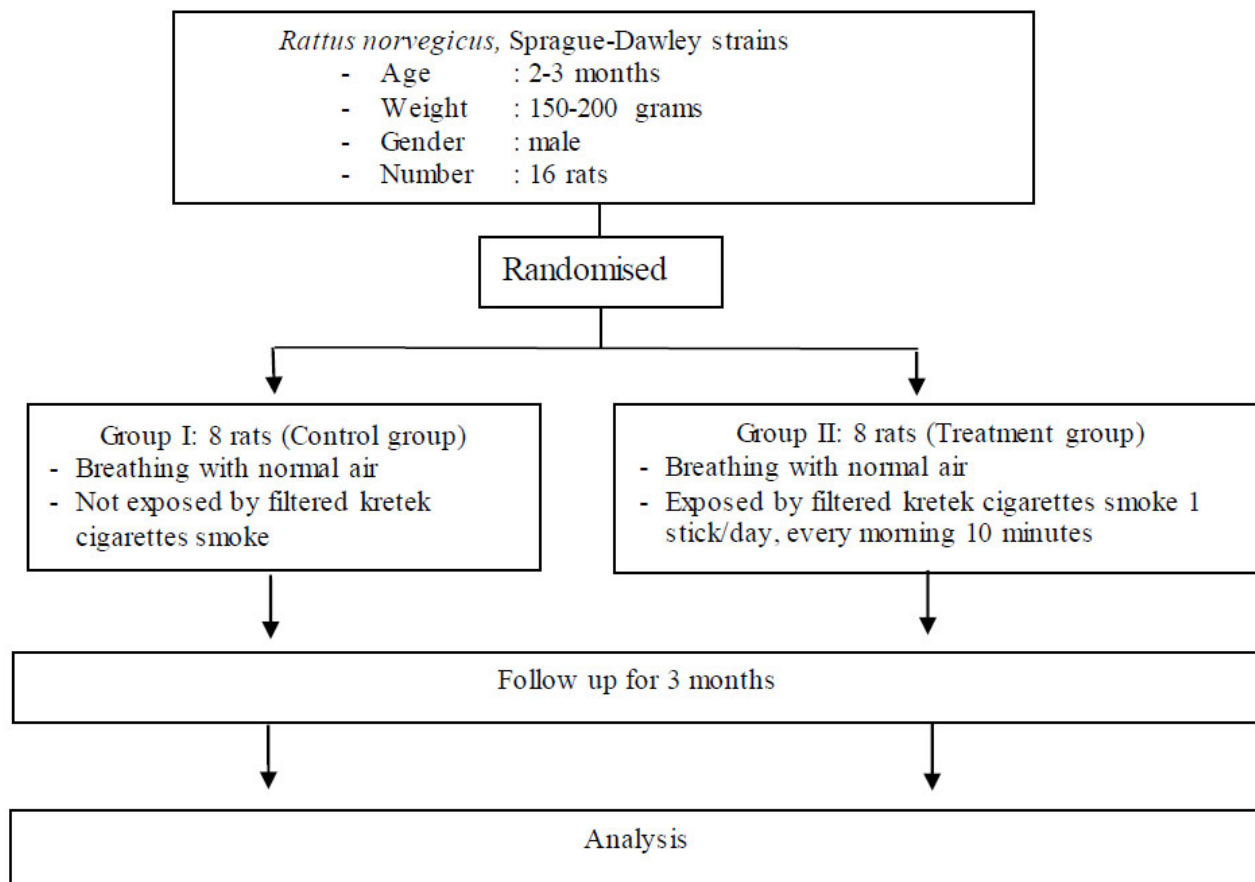
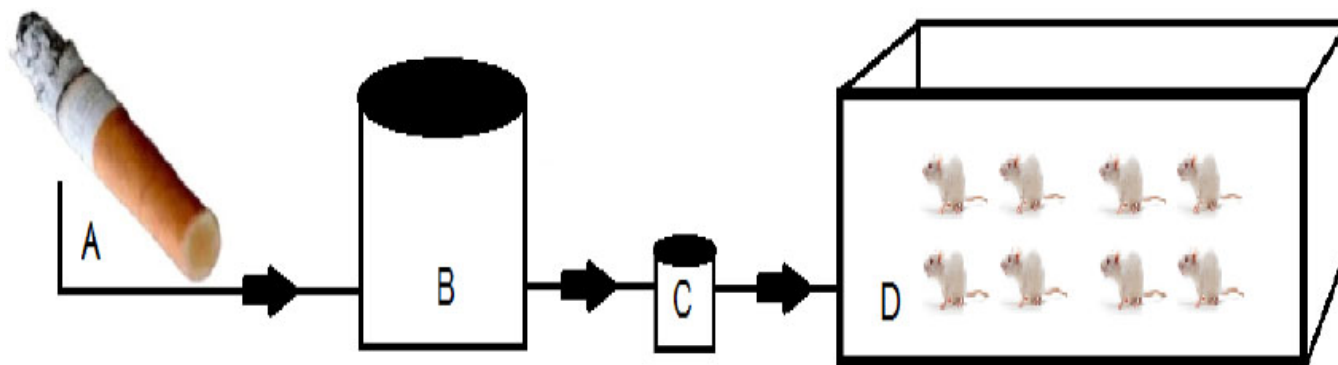


Fig. (1). Flowchart of experimental rat treatment.



**Fig. (2).** Schematic diagram of filtered kretek cigarettes smoke exposure to a groups of Sprague-Dawley rats. **A.** Filtered kretek cigarettes burned. **B.** Tube to accommodate filtered kretek cigarettes smoke. **C.** Electric pump. **D.** Glass box for rats treatment with length 40 cm, width 30 cm, and height 20 cm).

Acclimatization of rats was carried out for 2 weeks, then continued with treatment according to groups. Rats were divided into 2 groups with randomization. Group I was the rat group that was breathing normal air, and was not exposed to filtered kretek cigarette smoke (as a control). Group II, namely the rat group exposed to filtered kretek cigarettes smoke 1 stick/day/group (Fig. 2). The treatment duration for the two groups of rats was 3 months. After the treatment, the rats were sacrificed, and then their respiratory organs were collected from the trachea to the lungs to make histology slides.

**2.6. Measurement of Bronchioles and Respiratory Bronchioles**

The tissue was immersed in a neutral buffer solution of 10% formalin for 24 hours at room temperature. The tissue was cut to a size of 1x1x1 cm, and then put in a tissue cassette. The tissue was then transferred to alcohol dehydration with alcohol concentrations of 70%, 80%, 90%, and 96%, respectively. The dehydration time was 2 hours for each alcohol concentration. The next stage was clearing, after which, the tissue was ready to be inserted into the paraffin block. Furthermore, embedding and blocking were carried out. The tissue in the paraffin block was cut using a microtome with a thickness of 4-5 μ, and

then stained with hematoxylin and eosin.

Image documentation using Optilab Advance Plus and Image Raster 3 by PT MICONOS, Daerah Istimewa Yogyakarta, Indonesia; available at <https://miconos.ac.id/new/support/download>. Optilab Advance Plus and Image Raster 3 programs were used to analyze rat respiratory system histometrics (bronchioles and respiratory bronchioles) by three observers. Data for all groups were expressed as mean± standard deviation. Analyzing data between groups using an independent sample t-test. The difference between groups was significant if the test results showed P<0.05.

**2.7. Expression of p53 Gene in Rat Lung**

Measurement of p53 gene expression was carried out using real-time polymerase chain reaction (RT PCR). Lung tissue samples of Sprague Dawley rats were isolated for mRNA, and then the solution was put into a 1.5 mL tube, stored at -80 °C. The reagents for measuring mRNA levels were Bioline SensiFast Sybr Lo-ROX One-step kit, Zymo Research Quick-RNATM MiniPrep Plus, and Geneaid Micropestle. The p53 rat target gene used the sense primer (5' to 3') AGGCCTTGGAAGTCAAGGAT, while the antisense primer (5' to 3') TGAGTCAGGCCCTTCTGTCT, with a size of 140 bp. The glyceraldehyde-3-phosphate

**Table 1. Comparison of the effect of filtered kretek cigarette smoke on the Sprague Dawley rat lung.**

Sprague Dawley Rat Lung	Treatment Groups		p value
	Group I (n=8)	Group II (n=8)	
<b>-Bronchioles:</b>			
Length (μm)	40.42 ± 1.58	30.77 ± 0.78	0.000
Width (μm)	14.82 ± 0.41	9.28 ± 0.40	0.000
Area (μm <sup>2</sup> )	494.61 ± 5.62	297.32 ± 2.53	0.000
Perimeter (μm)	233.87 ± 4.51	177.84 ± 5.15	0.000
<b>-Respiratory bronchioles</b>			
Area (μm <sup>2</sup> )	17.68 ± 0.49	19.28 ± 0.38	0.000
Perimeter (μm)	26.60 ± 0.52	29.28 ± 0.35	0.000
<b>-Expression of p53 gene</b>	1 ± 0.00	3.02 ± 0.69	0.000

**Abbreviations:** Group I, rats breathe using ordinary air without exposure to filtered kretek cigarette smoke. Group II, the group of rats exposed to filtered kretek cigarette smoke 1 stick/day for 3 months of treatment.

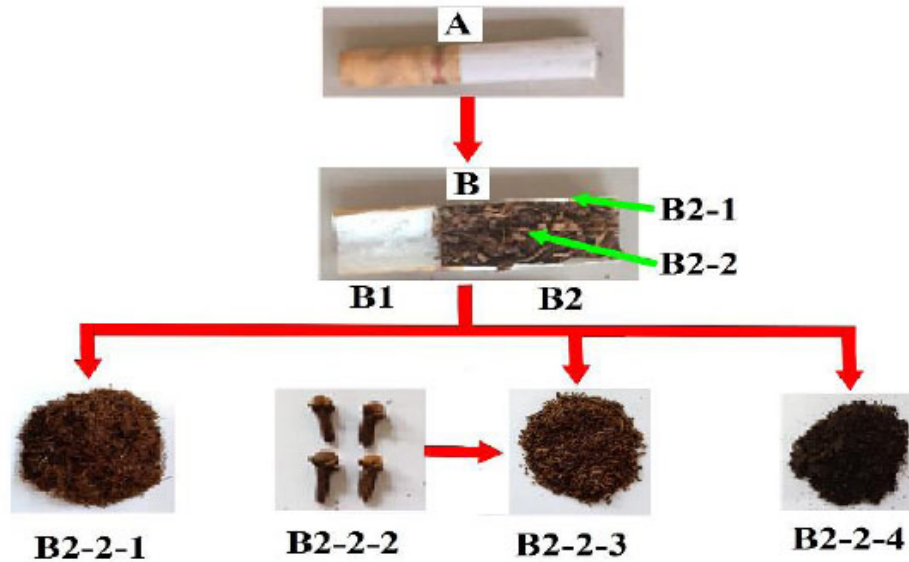
dehydrogenase (GAPDH) gene was used as an internal control. The sense primer of GAPDH gene was (5' to 3') CCAGGTGGTCTCCTCTGACTTCTC, while the antisense primer was (5' to 3') ATACCAGGAAATGAGCTTGACA, with a size of 147 bp [25]. RT PCR analysis using SYBR Green Master Mix (Bio-rad) in the Bio-rad CFX96 RT PCR system. Ct p53 gene was normalized with Ct gene GAPDH. The calculation was based on the  $2^{-\Delta\Delta C_T}$  method (Livak method)

[26], as demonstrated in Table 1.

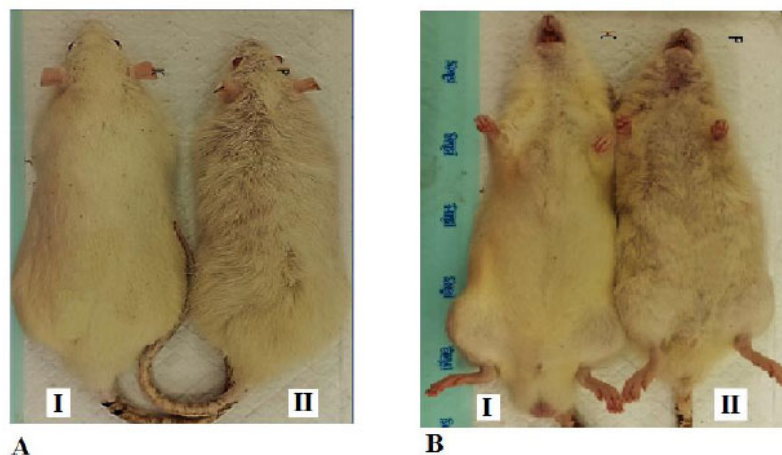
### 3. RESULTS

#### 3.1. Filtered Kretek Cigarettes

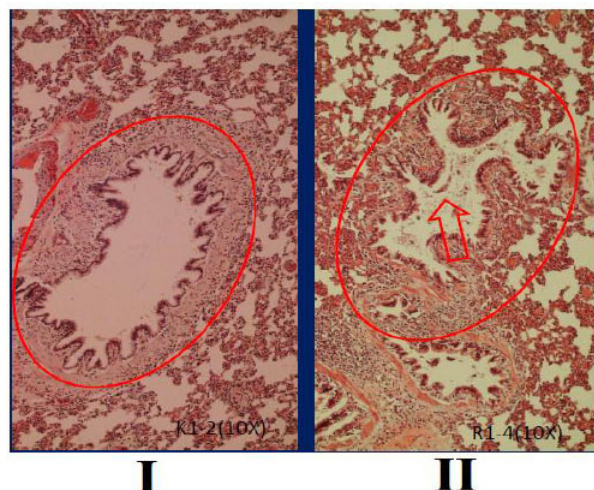
The constituent materials of filtered kretek cigarettes are presented in Fig. (3). Filtered kretek cigarettes consist of filters and kretek dough. Tobacco is the main ingredient of kretek cigarettes, which is mixed with cloves and sauce.



**Fig. (3).** Composition of filtered kretek cigarettes. (A). The appearance of filtered kretek cigarettes. (B). Filtered kretek cigarettes split lengthwise so that it appears the inside which consists of the filter (B1) and kretek section (B2). B2-1. Wrapping paper for filtered kretek cigarettes dough. B2-2-1. Tobacco as the main constituent of filtered kretek cigarettes dough. B2-2-2. Dried clove flower. B2-2-3. Chopped dried clove flower. B2-2-4. Kretek sauce.



**Fig. (4).** Hair of the Sprague-Dawley rats. **A.** Hair appearance of the back body. **B.** Hair appearance of the abdomen. I. Group I, rats breathe using ordinary air without exposure to filtered kretek cigarette smoke. II. Group II, the group of rats exposed to filtered kretek cigarette smoke 1 stick/day for 3 months of treatment.



**Fig. (5).** Bronchioles histological of Sprague-Dawley rats, stained with hematoxylin and eosin, objective 10x. I. Group I, rats breathe using ordinary air without exposure to filtered kretek cigarette smoke. II. Group II, the group of rats exposed to filtered kretek cigarette smoke 1 stick/day for 3 months of treatment. The red arrows showed mucus.

### 3.2. Hair Appearance

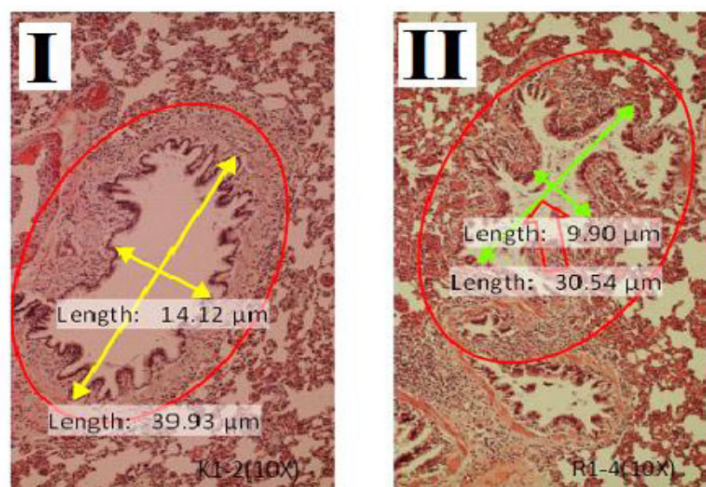
The hair appearance of rats between group I and group II is presented in Fig. (4). The hair on the back and abdomen of rats in group I looks white, clean, and dense, while in group II, it looks dull yellow, less clean, and sparse.

### 3.3. Bronchioles Histometric

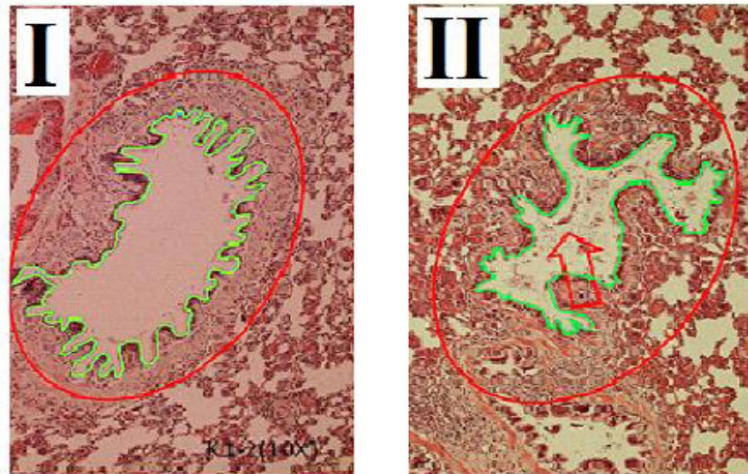
Histological of bronchioles in Sprague-Dawley rats between group I and group II is presented in Fig. (5). Treatment of filtered kretek cigarettes smoke 1 stick/day for 3 months causes mucus in the bronchioles space of rats in group II, while in group I, it is not found. In addition, the size of the bronchioles space also appears to be shrinking.

A comparison of bronchioles histometric in Sprague-

Dawley rats between group I and group II is presented in Fig. (6). Based on Fig. (6), seen that bronchioles length, width, area, and perimeter in group I are different compared to group II ( $P=0.000$ ). Bronchioles histometric of rats in group II  $< I$  ( $P=0.000$ ). In this study, the group of rats exposed to filtered kretek cigarette smoke had changes in bronchioles histometric, namely the decrease in length, width, area, and perimeter. The bronchioles length of rats in group II decreased by 24.14% compared to group I, while the bronchioles width of rats in group II decreased by 37.38% compared to group I. The bronchioles area of rats in group II decreased by 39.89% compared to group I, while the bronchioles perimeter of rats in group II decreased by 23.96% compared to group I. Rats in group II exert a negative response against filtered kretek cigarette smoke in the form of a decrease in the size of bronchioles histometric.



A



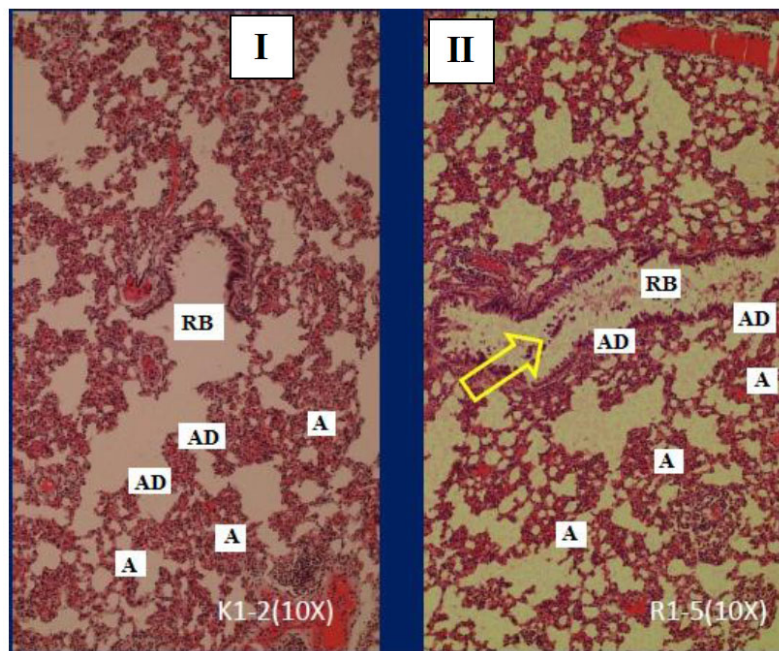
B

**Fig. (6).** Comparison of bronchioles histometric in Sprague-Dawley rats between group I compared to group II, staining with hematoxylin and eosin, objective10x. (A). Length and width of the bronchioles. (B). Area and perimeter of the bronchioles. I. Group I, rats breathe using ordinary air without exposure to filtered kretek cigarette smoke. II. Group II, the group of rats exposed to filtered kretek cigarette smoke 1 stick/day for 3 months of treatment. The red arrows showed mucus.

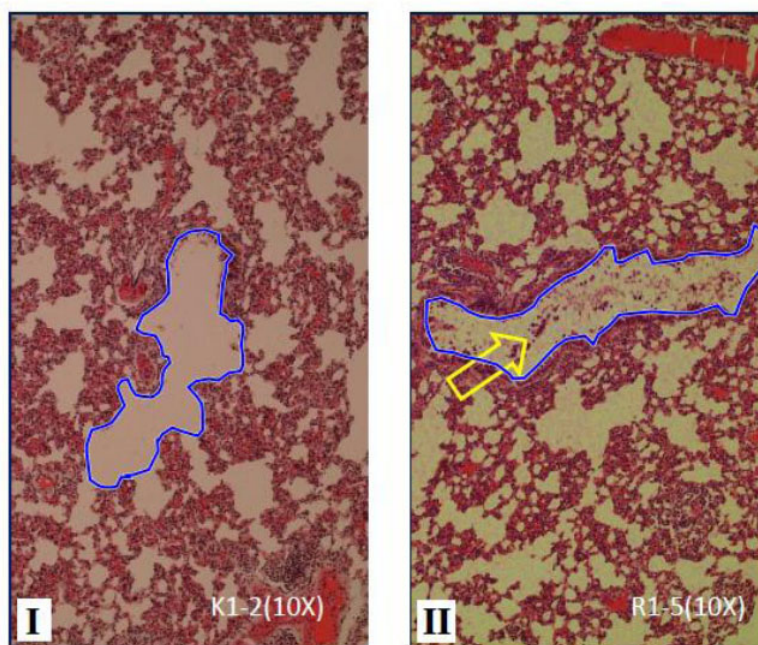
### 3.4. Respiratory Bronchioles Histometric

The histological of respiratory bronchioles in Sprague-Dawley rats between group I and group II is presented in

Fig. (7). Based on Fig. (7) treatment of filtered kretek cigarettes smoke 1 stick/day for 3 months causes mucus in the respiratory bronchioles space of Sprague-Dawley rats in group II, while in group I is not found.



**Fig. (7).** The histological of respiratory bronchioles in Sprague-Dawley rats between group I compared to group II, stained with haematoxylin and eosin, objective 10x. The yellow arrows showed mucus. RB= respiratory bronchioles, AD= alveolar ducts, A=alveoli. I. Group I, rats breathe using ordinary air without exposure to filtered kretek cigarette smoke. Group II, the group of rats exposed to filtered kretek cigarette smoke 1 stick/day for 3 months of treatment.



**Fig. (8).** Comparison of area and perimeter of respiratory bronchioles in Sprague-Dawley rats between group I compared to group II, stained with haematoxylin and eosin, objective 10x. Respiratory bronchioles of rats in group II showed mucus (yellow arrow), while in the group I mucus was not found. I. Group I, rats breathe using ordinary air without exposure to filtered kretek cigarette smoke. II. Group II, the group of rats exposed to filtered kretek cigarette smoke 1 stick/day for 3 months of treatment.

The area and the perimeter of respiratory bronchioles in the Sprague-Dawley rats between group I and group II is presented in Fig. (8). Fig. (8) demonstrated that the area and the perimeter of respiratory bronchioles of rats in group I < group II ( $P=0.000$ ). In this study, the group of rats exposed to filtered kretek cigarette smoke had changes in respiratory bronchioles histometric, namely the increase in area and perimeter. The area of respiratory bronchioles of rats in group II increased by 9.05% compared to group I, while the perimeter of respiratory bronchioles of rats in group II increased by 10.075% compared to group I. Rats in group II had a positive response against filtered kretek cigarette smoke in the form of an increase in the area and perimeter of respiratory bronchioles.

### 3.5. Expression of p53 Gene

Optimization of RT PCR for the determination of mRNA levels for p53 is presented in Fig. (9). Based on the measurement data, it was demonstrated that the treatment of rats exposed to filtered kretek cigarette smoke 1 cigarette/day for 3 months influenced p53 gene expression. There was an increase in p53 gene expression in rats exposed to filtered kretek cigarette smoke. It was proven that the mRNA levels of group II were higher (upregulation) than group I ( $P=0.000$ ).

## 4. DISCUSSION

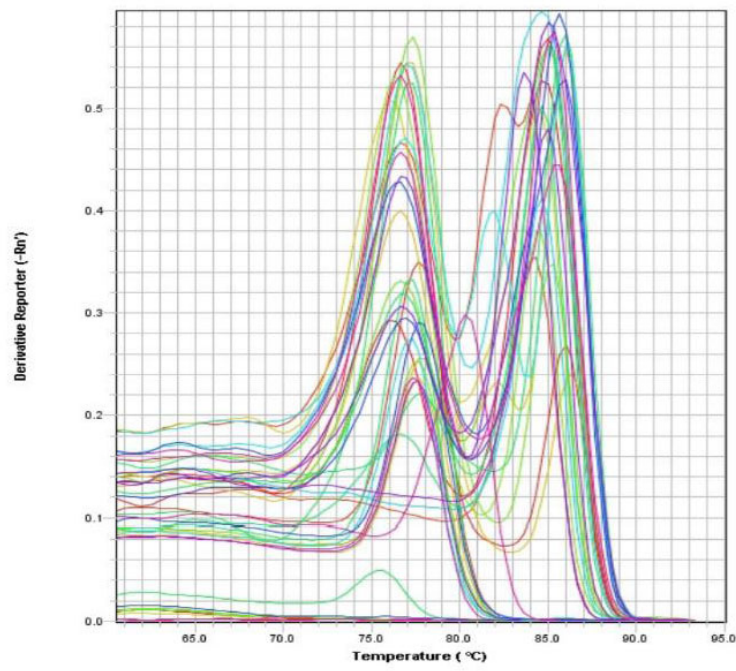
The color of the filter on filtered kretek cigarettes before burning was different from after burning. Before

the filtered kretek cigarettes were burned, the filter was white, while after they were burned, the filter had a burlywood color. It has been demonstrated that the function of the filter at the end of the filtered kretek cigarettes is to reduce tar and nicotine levels that enter the respiratory tract. The color change of the burlywood filter in this study indicated the presence of tar and nicotine from filtered kretek cigarettes. This fact is in line with previous research [27].

Filtered kretek cigarettes that were burning produced cigarette smoke and ash. Cigarette smoke was inhaled by rats, while ash of filtered kretek cigarettes was disposed of. Most of the filtered kretek cigarette smoke entered the respiratory system of rats. Filtered kretek cigarette smoke is one of the incomplete combustion products of tobacco, cloves, and sauce. Cigarette smoke includes main cigarette smoke and side cigarette smoke. Main cigarette smoke is cigarette smoke that was inhaled by rats as a model of active smokers. Side cigarette smoke is cigarette smoke that was in the environment around rats. In this study, filtered kretek cigarettes burned, and a pump mainly inhaled cigarette smoke and then channeled into the smoking box. This is similar to the smoking process that is generally carried out by smokers. The results of this study clearly show that there are differences in the color of the filters before and after the filtered kretek cigarettes burned. This fact clarifies the filter function of the filtered kretek cigarettes, which is to filter particles in cigarette smoke that are inhaled by smokers. Therefore, the filter on filtered kretek cigarettes can reduce the

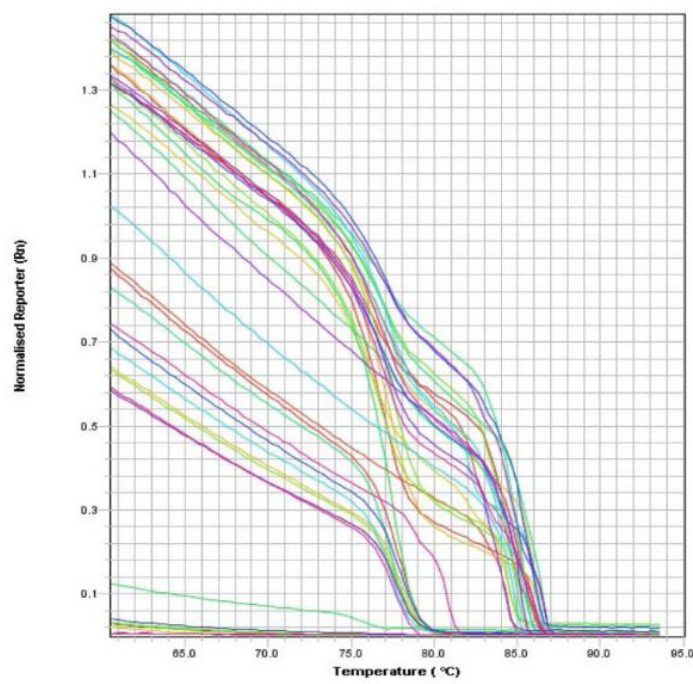


Melt Curve (Derivative Reporter)



A.

Melt Curve (Normalized Reporter)



B.

Fig. (9). Optimization of RT PCR for p53 gene expression. (A). Melting curve (derivative curve); (B). Melting curve (normalized reporter).

content of substances inhaled when smoking. Substances in cigarette smoke that have been shown to be high in content include nicotine, various metals [28], free radicals [29], oxidants and antioxidants [30]. Even though there is a filter, cigarette smoke in this study was proven to affect the hair appearance of rats, which worsened hair appearance.

Exposure to filtered kretek cigarette smoke in this study caused the hair of rats to change from pure white to dull yellow. The hair discoloration occurred throughout the rat's body. The results in this study were in accordance with the results of the report which stated that the smoker's mustache hair turned yellow [31]. Filtered kretek cigarette smoke in this study mostly comes from tobacco leaves, so it has nicotine. Nicotine in the filtered kretek cigarette smoke enters the body and then causes discoloration, cleanliness, and density of hair in the rats. This fact is in accordance with the results of earlier studies, which show that the nicotine content of hair is used as a biomarker to assess chronic exposure to environmental tobacco smoke [32]. Nicotine in cigarette smoke by inhalation enters the body and penetrates cell membranes to the systemic circulation system. It is further stated that nicotine is the main constituent of tobacco. Nicotine is not carcinogenic, but it triggers many carcinogens in tobacco [33]. Previous research has demonstrated that nicotine levels in the hair of smokers are much higher compared to non-smokers [34]. In addition to nicotine content, cigarette smoke also causes oxidative stress that affects the condition of the hair and, among others, affects the hair fiber [35]. In addition to affecting hair color, cigarette smoke also affects histometrics in the lungs of rats, including bronchioles and respiratory bronchioles.

The reduction in length, width, area, and perimeter of bronchioles in rats in this study responded to unwanted cigarette smoke exposure because, in normal conditions, the respiratory air does not contain cigarette smoke. This occurs as a defense mechanism for reducing the volume of cigarette smoke exposure. Reduction of bronchioles histometric reduces exposure to filtered kretek cigarette smoke, next to reducing the volume of cigarette smoke until it reaches the alveolar sacs (alveolar saccules). This situation allows obstruction of airflow that cannot flow back completely. This fact is consistent with earlier studies showing that the bronchioles are the site of airway obstruction. In addition to bronchioles abnormalities due to cigarette smoke, it was found that cigarette smoke also causes COPD [36]. Based on the facts obtained, we agree that pulmonary histometrics is useful for quantitatively measuring the tissues that make up the organs in the respiratory system. Moreover, it has been demonstrated that exposure to filtered kretek cigarettes at a dose of 1 cigarette/day or 2 cigarettes/day for 30 days, causes bronchial mucosal hyperplasia and bronchoconstriction in male Sprague Dawley rats [37]. Apart from that, it has also been demonstrated that filtered kretek cigarette smoke not only impacts the respiratory organs but also impacts Purkinje cells and pyramidal cells in Sprague Dawley rats [38].

In addition, the facts in this study, in accordance with the results of other studies which, indicated that structural abnormalities and blockage by mucus can cause destabilization of the airways in the bronchioles [39].

Previous studies have shown that morphometric measurements of rat lungs have been carried out [17]. In addition to the morphometric analysis of the rat lungs, pulmonary histometrics have also been shown. The results of the study indicated that the diameter of the respiratory bronchioles, alveolar ducts, and alveoli were  $24.93 \pm 1.27 \mu\text{m}$ ,  $21.14 \pm 0.66 \mu\text{m}$ , and  $12.95 \pm 0.21 \mu\text{m}$ , respectively [18]. Need to note that it is not only cigarette smoke that causes chronic obstruction of the bronchioles. It has been shown that chronic obstruction of the bronchioles can also be caused by cell degeneration in the bronchioles [40]. Therefore, it is still necessary to prove the effect of cigarette smoke on cell death in the bronchioles.

The effect of filtered kretek cigarette smoke in this study caused the area and perimeter of respiratory bronchioles in group II of rats to be greater compared to group I as a control. The effect of filtered kretek smoke in this study narrowed the bronchioles space (Fig. 6) but widened the respiratory bronchioles space (Fig. 8). The increased respiratory bronchioles space may be due to the pressure of filtered kretek cigarette smoke, such as the alveolar sac. The changes in histometric in the respiratory bronchioles in this study are in line with the other studies showing significant changes in the respiratory bronchioles of rats after treatment with hypoxia and melatonin [41].

Our results showed that mucus appeared in the bronchioles and respiratory bronchioles of rats. Filtered kretek cigarette smoke 1 cigarette/day for 3 months caused mucus in the respiratory bronchioles space in group II, while in group I (control), this mucus was not found (Figs. 5-8). Of note, apart from in the bronchioles and respiratory bronchioles, mucus is also found in the saccus alveolar space (data not shown). The mucus is a complex mixture of mucins. Mucins are produced by airway epithelial cells and submucosal glands [42]. As a result of mucus that has accumulated in the bronchioles and respiratory bronchioles, airflow in the lungs is limited, thus resulting in lung disorders. In addition, demonstrated that inflammatory cells produce excess mucus which reduces lung function [43].

Previous studies demonstrated that cigarette smoke causes oxidative stress due to an imbalance between oxidants and antioxidants. Furthermore, cigarette smoke also induces epithelial cells for mucus hypersecretion, increasing proinflammatory cytokines and chemokines. Cigarette smoke has also been shown to increase macrophages and neutrophils and disrupt the balance of lymphocytes [30]. Cigarette smoke causes mucus hypersecretion and COPD. In addition, exposure to cigarette smoke can worsen COPD [44]. The results of other studies have also demonstrated that smoking at a young age is a dominant factor in the cause of COPD [45]. Based on the results of this study and several other studies, it is clear that cigarette smoke has a complex effect on lung tissue [46]. Moreover, cigarette smoke induces epigenetic changes in lung tissue [47], lung cancer [48, 49], oral disease, myocardial infarction, oral cancer, chronic health disease, cardiovascular disease, and asthma [50].

The Ct of the p53 gene for group I is 1, while the Ct p53 gene for group II shows a multiple of 1, so it experiences upregulation [51]. Of the 8 samples in this study, all showed up-regulation, and none showed downregulation. Rats exposed to filtered kretek cigarette smoke showed increased lung mRNA levels of the p53 gene. The occurrence of up regulation of the p53 gene in mice exposed to low doses of filtered kretek cigarette smoke in this study is in accordance with the statement relating to the processes of proliferation, apoptosis, and metabolism to suppress tumorigenesis [24]. It was further stated that the p53 gene plays an important role in responding to various types of stress cells [52]. The results of this study can be used as a basis for further research on tobacco consumption (cigarettes) with the occurrence of lung cancer. This is in accordance with research results, which demonstrate that tobacco use and variations in the p53 gene increase the risk of oral cancer [53, 54].

## 5. LIMITATIONS

The present study has limitations; among others, we did not measure the levels of chemical compounds in filtered kretek cigarette smoke. In this study, we have not determined damage to the bronchioles and respiratory bronchioles based on **histometrics due** to exposure to filtered kretek cigarette smoke. In addition, we have not performed cytometrics on bronchioles and respiratory bronchioles as we did before with human cervical cells [54, 55]. We hope that in the next opportunity, we can demonstrate the relationship between the levels of chemical compounds in filtered Kretek cigarette smoke with the level of the bronchioles and respiratory bronchioles damage based on cytometric and histometric.

## CONCLUSION

Based on the results obtained, we concluded that Sprague-Dawley rats in group II were exposed to 1 stick/day of filtered kretek cigarette smoke for 3 months and showed dull yellow hair on the back and abdomen, less clean, and less frequent compared **to the group** I (control). The size of bronchioles of Sprague-Dawley rats decreased after being exposed to filtered kretek cigarettes smoke 1 stick/day for 3 months, while the size of **respiratory bronchioles** increased. Mucus is found in the bronchioles and **respiratory bronchioles** of Sprague-Dawley rats. Mucus was found in the bronchioles, and **respiratory bronchioles** in the group were exposed to 1 stick/day of filtered kretek cigarette smoke for 3 months, while in the control group, there was no visible mucus. It is clear that the quantitative changes in bronchioles and respiratory bronchioles are caused by the effects of filtered kretek cigarettes smoke. Quantitative changes in the bronchioles and respiratory bronchioles are important so that the diagnosis related to lung tissue disorders can be determined more objectively. In addition, it was also shown that exposure to low doses of filtered kretek cigarette smoke, 1 cigarette/day for 3 months, increased the expression of the p53 gene (**upregulation**) in the rat lungs.

## SIGNIFICANCE STATEMENT

This study discovered that exposure to low-dose filtered kretek cigarette **smoke changed the histometric of bronchioles and respiratory bronchioles** in the Sprague-Dawley rats model. In addition, exposure to low-dose filtered kretek cigarette smoke also showed an increase in p53 gene expression in rat lungs.

## LIST OF ABBREVIATIONS

COPD = Chronic Obstructive Pulmonary Disease  
 RT PCR = Real-time Polymerase Chain Reaction  
 mRNA = Messenger ribonucleic acid  
 GAPDH = Glyceraldehyde-3-phosphate dehydrogenase

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The ethical clearance of this research was obtained from the Research Ethics Commission, Faculty of Medicine, Universitas Trisakti, Jakarta, Indonesia (No. 184/KER-FK/VIII/2018).

## HUMAN AND ANIMAL RIGHTS

No humans were used for studies that are the basis of this study. This study adheres to internationally accepted standards for animal research, following the 3Rs principle. The ARRIVE guidelines were employed for reporting experiments involving live animals, promoting ethical research practices.

## CONSENT FOR PUBLICATION

Not applicable.

## AVAILABILITY OF DATA AND MATERIAL

All the data and supporting information is provided within the article.

## FUNDING

This work is supported by the Faculty of Medicine, Universitas Trisakti, Indonesia. This research funding is in the budget year 2019-2020.

## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

## ACKNOWLEDGEMENTS

Declared none.

## REFERENCES

- [1] Kasri R, Ahsan A, Wiyono NH, Jacinda A, Kusuma D. New evidence of illicit cigarette consumption and government revenue loss in Indonesia. *Tob Induc Dis* 2021; 19: 1-8. <http://dx.doi.org/10.18332/tid/142778> PMID: 34782828
- [2] WHO. Tobacco. 2020. Available From: <https://www.who.int/news-room/fact-sheets/detail/tobacco>
- [3] Jha P. The hazards of smoking and the benefits of cessation: A critical summation of the epidemiological evidence in high-income countries. *eLife* 2020; 9: e49979. <http://dx.doi.org/10.7554/eLife.49979> PMID: 32207405

- [4] SEATCA (Southeast Asia Tobacco Control Alliance). Tobacco Control Atlas ASEAN Region. 2018. Available From: [https://seatca.org/dmdocuments/TobaccoControlAtlas\\_ASEANRegion\\_4thEd\\_Dec2019.pdf](https://seatca.org/dmdocuments/TobaccoControlAtlas_ASEANRegion_4thEd_Dec2019.pdf)
- [5] Palipudi K, Mbulo L, Kosen S, et al. A cross sectional study of kretek smoking in Indonesia as a Major risk to public health. Asian Pac J Cancer Prev 2015; 16(16): 6883-8. <http://dx.doi.org/10.7314/APJCP.2015.16.16.6883> PMID: 26514461
- [6] The Indonesian Sweetener and Fiber Crops Research Institute (ISFCRI). Tobacco 2015. Available From: <http://balittas.litbang.pertanian.go.id/index.php/en/komoditas/tobacco>
- [7] ITIS (Integrated Taxonomi Information System). *Syzygium aromaticum* (L.) Merr. & L.M. Perry. Taxonomic Serial No.: 506167. 2020. Available From: <https://www.itis.gov/servlet/SingleRpt/SingleRpt/>
- [8] Arnez M. Tobacco and Kretek: Indonesian drugs in historical change. 2009. Available From: <https://nbn-resolving.org/urn:nbn:de:0168-ssoar-362761>
- [9] Lin J, Taggart M, Borthwick L, et al. Acute cigarette smoke or extract exposure rapidly activates TRPA1-mediated calcium influx in primary human airway smooth muscle cells. Sci Rep 2021; 11(1): 9643. <http://dx.doi.org/10.1038/s41598-021-89051-4> PMID: 33953304
- [10] Amorós-Pérez A, Cano-Casanova L, Román-Martínez MC, Lillo-Ródenas MA. Comparison of particulate matter emission and soluble matter collected from combustion cigarettes and heated tobacco products using a setup designed to simulate puffing regimes. Chem Eng J Adv 2021; 8(100144): 100144. <http://dx.doi.org/10.1016/j.cej.2021.100144>
- [11] Braun M, Fromm EL, Gerber A, Klingelhöfer D, Müller R, Gronenberg DA. Particulate matter emissions of four types of one cigarette brand with and without additives: A laser spectrometric particulate matter analysis of secondhand smoke. BMJ Open 2019; 9(1): e024400. <http://dx.doi.org/10.1136/bmjopen-2018-024400> PMID: 30782733
- [12] Paul AR, Khan F, Jain A, Saha SC. Deposition of smoke particles in human airways with realistic waveform. Atmosphere (Basel) 2021; 12(7): 912. <http://dx.doi.org/10.3390/atmos12070912>
- [13] González-Luis GE, van Westering-Kroon E, Villamor-Martínez E, et al. Tobacco Smoking during pregnancy is associated with increased risk of Moderate/Severe Bronchopulmonary Dysplasia: A systematic review and meta-analysis. Front Pediatr 2020; 8(160): 160. <http://dx.doi.org/10.3389/fped.2020.00160> PMID: 32411634
- [14] WHO. WHO highlights huge scale of tobacco-related lung disease deaths. 2019. Available From: <https://www.who.int/news/item/29-05-2019-who-highlights-huge-scale-of-tobacco-related-lung-disease-deaths>
- [15] Carvalho JL, Miranda M, Fialho AK. Oral feeding with probiotic *Lactobacillus rhamnosus* attenuates cigarette smoke-induced COPD in C57Bl/6 mice: Relevance to inflammatory markers in human bronchial epithelial cells. PLoS One 2020; 15(4): e0225560. <http://dx.doi.org/10.1371/journal.pone.0225560>
- [16] Shraideh ZA, Najjar HN. Histological changes in tissues of trachea and lung alveoli of albino rats exposed to the smoke of two types of Narghile Tobacco Products. 2011. Available From: <http://ijbs.hu.edu.jo/FILES/v4n4/Paper%20Number%205.pdf>
- [17] Maina JN, Igbokwe CO. Comparative morphometric analysis of lungs of the semifossorial giant pouched rat (*Cricetomys gambianus*) and the subterranean Nigerian mole rat (*Cryptomys foxi*). Sci Rep 2020; 10(1): 5244. <http://dx.doi.org/10.1038/s41598-020-61873-8> PMID: 32251351
- [18] Ibe CS, Onyeausi BI, Salami SO, Nzalak JO. Microscopic anatomy of the lower respiratory system of the African Giant Pouched Rat (*Cricetomys gambianus*, Waterhouse 1840). Int J Morphol 2011; 29(1): 27-33. <http://dx.doi.org/10.4067/S0717-95022011000100004>
- [19] Serra DS, De Brito KBP, Oliveira KL. Respiratory system of rats exposed to pollutants arising out of heating residual glycerol. J Fundament Renewable Energy Appl 2017; 7(1): 1-4. <http://dx.doi.org/10.4172/2090-4541.1000220>
- [20] Wawryk-Gawda E, Chylińska-Wrzos P, Zarobkiewicz M, Chłapek K, Jodłowska-Jędrych B. Lung histomorphological alterations in rats exposed to cigarette smoke and electronic cigarette vapour. Exp Ther Med 2020; 19(4): 2826-32. <http://dx.doi.org/10.3892/etm.2020.8530> PMID: 32256766
- [21] Kim V, Criner GJ. Chronic bronchitis and chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2013; 187(3): 228-37. <http://dx.doi.org/10.1164/rccm.201210-1843CI> PMID: 23204254
- [22] Zuo WL, Yang J, Gomi K, Chao I, Crystal RG, Shaykhiyev R. EGF-amphiregulin interplay in airway stem/progenitor cells links the pathogenesis of smoking-induced lesions in the human airway epithelium. Stem Cells 2017; 35(3): 824-37. <http://dx.doi.org/10.1002/stem.2512> PMID: 27709733
- [23] Chauhan NS, Sood D, Takkar P, Dhadwal DS, Kapila R. Quantitative assessment of airway and parenchymal components of chronic obstructive pulmonary disease using thin-section helical computed tomography. Pol J Radiol 2019; 84: 54-60. <http://dx.doi.org/10.5114/pjr.2019.82737> PMID: 31019595
- [24] Fischer M. Census and evaluation of p53 target genes. Oncogene 2017; 36(28): 3943-56. <http://dx.doi.org/10.1038/onc.2016.502> PMID: 28288132
- [25] Petre CE, Sin SH, Dittmer DP. Functional p53 signaling in Kaposi's sarcoma-associated herpesvirus lymphomas: Implications for therapy. J Virol 2007; 81(4): 1912-22. <http://dx.doi.org/10.1128/JVI.01757-06> PMID: 17121789
- [26] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25(4): 402-8. <http://dx.doi.org/10.1006/meth.2001.1262> PMID: 11846609
- [27] Sarikaya Y, Kahya ND, Pekdemir AD, Önal M. Removing tar and nicotine from mainstream cigarette smoke using sepiolite-modified filter tips. Clay Miner 2020; 55(4): 375-82. <http://dx.doi.org/10.1180/clm.2021.7>
- [28] Wallin C, Sholtz SB, Österlund N, et al. Alzheimer's disease and cigarette smoke components: Effects of nicotine, PAHs, and Cd(II), Cr(III), Pb(II), Pb(IV) ions on amyloid- $\beta$  peptide aggregation. Sci Rep 2017; 7(1): 14423. <http://dx.doi.org/10.1038/s41598-017-13759-5> PMID: 29089568
- [29] Anggraeni NKN, Djoko DJHS, Juswono UP. Identification of free radical in main stream cigarette smoke in cigarette with clove mix (Kretek) and cigarette without clove mix (White). Buletin Fisika 2020; 212(73): 81.
- [30] Strzelak A, Ratajczak A, Adamiec A, Feleszko W. Tobacco smoke induces and alters immune responses in the lung triggering inflammation, allergy, asthma and other lung diseases: A mechanistic review. Int J Environ Res Public Health 2018; 15(5): 1033. <http://dx.doi.org/10.3390/ijerph15051033> PMID: 29883409
- [31] Laborada J, Cohen PR. Smoker's mustache revisited: Upper lip hair yellow discoloration associated with tobacco. Cureus 2021; 13(10): e18988. <http://dx.doi.org/10.7759/cureus.18988> PMID: 34820243
- [32] Nadhiroh SR, Djokosujono K, Utari DM, Hasugian AR. Questionnaire-based environmental tobacco smoke exposure and hair nicotine levels in 6-month-old infants: A validation study in Indonesia. Glob Pediatr Health 2020; 7: 2333794X2096928. <http://dx.doi.org/10.1177/2333794X20969287> PMID: 33195749
- [33] Murphy SE. Biochemistry of nicotine metabolism and its relevance to lung cancer. J Biol Chem 2021; 296(100722): 100722. <http://dx.doi.org/10.1016/j.jbc.2021.100722> PMID: 33932402
- [34] Inukai T, Kaji S, Kataoka H. Analysis of nicotine and cotinine in hair by on-line in-tube solid-phase microextraction coupled with liquid chromatography-tandem mass spectrometry as biomarkers

- of exposure to tobacco smoke. *J Pharm Biomed Anal* 2018; 156: 272-7.  
<http://dx.doi.org/10.1016/j.jpba.2018.04.032> PMID: 29729641
- [35] Trüeb RM. The impact of oxidative stress on hair. *Int J Cosmet Sci* 2015; 37(S2) (Suppl. 2): 25-30.  
<http://dx.doi.org/10.1111/ics.12286> PMID: 26574302
- [36] Kim T, Kang J. Association between dual use of e-cigarette and cigarette and chronic obstructive pulmonary disease: An analysis of a nationwide representative sample from 2013 to 2018. *BMC Pulm Med* 2021; 21(1): 231.  
<http://dx.doi.org/10.1186/s12890-021-01590-8> PMID: 34256746
- [37] Tjahyadi D, Parwanto E. Effects of low-dose filtered kretek cigarette smoke on bronchial smooth muscle in male Sprague-Dawley rats. *Univ Med* 2023; 42: 259-71.  
<http://dx.doi.org/10.18051/UnivMed.2023.v42:259-271>
- [38] Tjahyadi D, Parwanto E, Amalia H, Digambiro RA, Edy HJ, Oladimeji AV. Decreased density of pyramidal cells in the cerebral cortex, and Purkinje cells in the cerebellar cortex of Sprague-Dawley rats after being exposed to filtered kretek cigarette smoke. *J Bio Res* 2023; 96(1): 1-6.  
<http://dx.doi.org/10.4081/jbr.2023.10757>
- [39] Burgel P-R, Martin F, Frijalvo A. Dysfunctional lung anatomy and small airways degeneration in COPD. *Int J Chron Obstruct Pulmon Dis* 2013; 8: 7-13.  
<http://dx.doi.org/10.2147/COPD.S28290> PMID: 23319856
- [40] Chookliang A, Sricharoenvej S, Lanlua P. Alterations in the bronchioles of rat lungs with chronic diabetes. 2019. Available From:  
[http://conf.ejikei.org/ICTSS/2019/proceedings/materials/proc\\_files/GS/ICTSS2019\\_GS\\_A007/CameraReady\\_ICTSS2019\\_GS\\_A007\\_Ms.Chookliang.pdf](http://conf.ejikei.org/ICTSS/2019/proceedings/materials/proc_files/GS/ICTSS2019_GS_A007/CameraReady_ICTSS2019_GS_A007_Ms.Chookliang.pdf)
- [41] Yanko R, Levashov M, Chaka E, Safonov S. Morphofunctional state of the lungs respiratory part in normotensive and hypertensive rats after combined exposure to intermittent hypoxia and melatonin. *J Educ Health Sport* 2021; 11(1): 56-68.  
<http://dx.doi.org/10.12775/JEHS.2021.11.01.006>
- [42] Whitsett JA. Airway epithelial differentiation and mucociliary clearance. *Ann Am Thorac Soc* 2018; 15 (Suppl. 3): S143-8.  
<http://dx.doi.org/10.1513/AnnalsATS.201802-128AW> PMID: 30431340
- [43] Zhang H, Yu W, Ji L, *et al.* Guifu dihuang pills ameliorated mucus hypersecretion by suppressing Muc5ac expression and inactivating the ERK-SP1 pathway in lipopolysaccharide/cigarette smoke-induced mice. *Evid Based Complement Alternat Med* 2021; 2021: 1-15.  
<http://dx.doi.org/10.1155/2021/9539218> PMID: 34777538
- [44] Duan J, Cheng W, Zeng Y, *et al.* Characteristics of Patients with chronic obstructive pulmonary disease exposed to different environmental risk factors: A large cross-sectional study. *Int J Chron Obstruct Pulmon Dis* 2020; 15: 2857-67.  
<http://dx.doi.org/10.2147/COPD.S267114> PMID: 33192059
- [45] Safitri W, Martini S, Artanti KD, Li CY. Smoking from a younger age is the dominant factor in the incidence of chronic obstructive pulmonary disease: Case-control study. *Int J Environ Res Public Health* 2021; 18(11): 6047.  
<http://dx.doi.org/10.3390/ijerph18116047> PMID: 34199771
- [46] Seton-Rogers S. Complex effects of tobacco on lung tissue. *Nat Rev Cancer* 2020; 20(4): 199.  
<http://dx.doi.org/10.1038/s41568-020-0249-y> PMID: 32112043
- [47] Seiler CL, Song JM, Kotandeniya D, *et al.* Inhalation exposure to cigarette smoke and inflammatory agents induces epigenetic changes in the lung. *Sci Rep* 2020; 10(1): 11290.  
<http://dx.doi.org/10.1038/s41598-020-67502-8> PMID: 32647312
- [48] Aredo JV, Luo SJ, Gardner RM, *et al.* Tobacco smoking and risk of second primary lung cancer. *J Thorac Oncol* 2021; 16(6): 968-79.  
<http://dx.doi.org/10.1016/j.jtho.2021.02.024> PMID: 33722709
- [49] Vinay S, Dharmashekara C, Prasad A. Smoking carcinogens and lung cancer-a review. *Asian J Pharm Clin Res* 2021; 14(1): 5-12.  
<http://dx.doi.org/10.22159/ajpcr.2021v14i1.39811>
- [50] Nuryunarsih D, Lewis S, Langley T. Health risks of Kretek Cigarettes: A systematic review. *Nicotine Tob Res* 2021; 23(8): 1274-82.  
<http://dx.doi.org/10.1093/ntr/ntab016> PMID: 33503247
- [51] Sadia H, Ahmad Bhinder M, Irshad A, *et al.* Determination of expression profile of p53 gene in different grades of breast cancer tissues by real time PCR. *Afr Health Sci* 2020; 20(3): 1273-82.  
<http://dx.doi.org/10.4314/ahs.v20i3.32> PMID: 33402975
- [52] Shi D, Jiang P. A different facet of p53 Function: Regulation of immunity and inflammation during tumor development. *Front Cell Dev Biol* 2021; 9(762651): 762651.  
<http://dx.doi.org/10.3389/fcell.2021.762651> PMID: 34733856
- [53] Singh R, Patel K, Patel J, Patel P. Association of interactions between Metabolic 'Caretaker' Genes, p53, MDM2, and Tobacco Use with the Risk of Oral Cancer: A multifactor dimensionality reduction approach. *Asian Pac J Cancer Prev* 2023; 24(4): 1231-7.  
<http://dx.doi.org/10.31557/APJCP.2023.24.4.1231> PMID: 37116145
- [54] Parwanto MLE, Wratsangka R, Guyansyah A, Anggraeni K. Mutation of the Fas-promoter-670 gene, AA to GA in the normal cervix-epithelial-cells of high risk Indonesian mother: A case report. *Bali Med J* 2019; 8(1): 360-4.  
<http://dx.doi.org/10.15562/bmj.v8i1.1313>
- [55] Parwanto E, Wratsangka R, Guyansyah A, *et al.* The change of cell biometric and its nucleus on cervical-squamous-epithelial-cell with GA genotype of Fas-promoter-670 gene, high-risk human papillomavirus and *Candida* species infection: A case report. *Bali Med J* 2021; 10(1): 74-81.  
<http://dx.doi.org/10.15562/bmj.v10i1.2138>

**DISCLAIMER:** The above article has been published, as is, ahead-of-print, to provide early visibility but is not the final version. Major publication processes like copyediting, proofing, typesetting and further review are still to be done and may lead to changes in the final published version, if it is eventually published. All legal disclaimers that apply to the final published article also apply to this ahead-of-print version.

---

## Thankyou For Uploading Proof Corrections | BMS-TORMJ-2023-19

---

**Edy Parwanto** <edyparwanto@trisakti.ac.id>

Sat, Apr 27, 2024 at 6:25 AM

To: YUSRA Junani <yusra@benthamcorrespondenceteam.com>

Dear Editorial Team,  
Please allow me to send the final corrected manuscript.  
Thank You,  
Regards  
Edy Parwanto  
[Quoted text hidden]

---

 **BMS-TORMJ-2023-19-PROOF READ-2024-04-26.pdf**  
2465K

---

## Thankyou For Uploading Proof Corrections | BMS-TORMJ-2023-19

---

**Edy Parwanto** <edyparwanto@trisakti.ac.id>

Mon, Apr 29, 2024 at 6:08 PM


To: YUSRA Junani <yusra@benthamcorrespondenceteam.com>

Dear Yusra Junani  
Editorial Manager  
Bentham Science Publishers

I am having difficulty sending the article BMS-TORMJ-2023-19 which I have reviewed and is available on my dashboard. Allow me to send the article via email (attached), please include it in my dashboard.

Regards,  
Edy Parwanto  
[Quoted text hidden]

---

 **BMS-TORMJ-2023-19-(5)-PROOF READ-2024-04-26.pdf**  
2465K

---

## Thankyou For Uploading Proof Corrections | BMS-TORMJ-2023-19

---

**YUSRA Junani** <yusra@benthamcorrespondenceteam.com>

Tue, Apr 30, 2024 at 3:21 PM

To: Edy Parwanto <edyparwanto@trisakti.ac.id>

Cc: Ambreen Irshad <ambreenirshad@benthamsience.net>, Sobia Adnan <sobiaadnan@benthamsience.net>

Dear Dr. Parwanto,

Apologies.

We have safely received the attached proof corrections for your article Refrence number **BMS-TORMJ-2023-19** and forwarded it to the concerned department to be uploaded on our online submission system on your behalf.

Please feel free to contact me in case of any further queries.

Best Regards,


**Yusra Junani**  
**Editorial Manager**  
**Bentham Science Publishers**

[Quoted text hidden]



# Low Doses of Kretek Cigarette Smoke Altered Rat Lung Histometric, and Overexpression of the p53 Gene



Edy Parwanto<sup>1,\*</sup> , David Tjahyadi<sup>2</sup>, Sisca Sisca<sup>1</sup>, Husnun Amalia<sup>3</sup>, Nany Hairunisa<sup>4</sup>, Hosea Jaya Edy<sup>5</sup>, Ashaolu Victoria Oladimeji<sup>6</sup> and Noureddine Djebli<sup>7</sup>

<sup>1</sup>Department of Biology, Faculty of Medicine, Universitas Trisakti, Jl. Kyai Tapa, Kampus B, No.260 Grogol 11440, Jakarta, Indonesia

<sup>2</sup>Department of Histology, Faculty of Medicine, Universitas Trisakti, Jakarta, Indonesia

<sup>3</sup>Department of Ophthalmology, Faculty of Medicine, Universitas Trisakti, Jakarta, Indonesia

<sup>4</sup>Department of Occupational Medicine, Faculty of Medicine, Universitas Trisakti, Jakarta, Indonesia

<sup>5</sup>Study Program of Pharmacy, Faculty of Math, and Natural Sciences, Universitas Sam Ratulangi, Manado, Indonesia

<sup>6</sup>Department of Chemistry, Loyola Institute of Frontier Energy, Loyola College, Chennai, India

<sup>7</sup>Department of Biologie, Faculty of Natural and Life Sciences, Abdelhamid Ibn Badis University, Mostaganem, Algeria

## Abstract:

**Background:** The components of kretek cigarettes include tobacco as the main part, clove, and sauce. Filtered kretek cigarettes are kretek cigarettes that have one end filtered. Cigarette smoke contributes to the disruption of the respiratory system, so it is necessary to know the effect of low doses of cigarette smoke on changes in the histometric of the respiratory system, and whether it affects p53 gene expression. This study aims to determine changes in the histometric of the respiratory system and p53 gene expression.

**Methods:** In this study, we used Sprague-Dawley rats. Group I of rats breathing normal air, were not exposed to filtered kretek cigarette smoke (as a control). Group II of rats, as a treatment group, were exposed to filtered kretek cigarette smoke 1 stick/day for 3 months. The results of lung histometry measurements and p53 gene expression between groups were analyzed using the Independent Sample T-test. The difference between groups is significant if the test results show  $P < 0.05$ .

**Results:** Bronchioles length, width, area, and perimeter in group I were  $40.55 \pm 1.57 \mu\text{m}$ ,  $14.82 \pm 0.41 \mu\text{m}$ ,  $494.61 \pm 5.62 \mu\text{m}^2$ , and  $233.87 \pm 4.51 \mu\text{m}$ , respectively. Bronchioles length, width, area, and perimeter in group II were  $30.76 \pm 0.78 \mu\text{m}$ ,  $9.28 \pm 0.40 \mu\text{m}$ ,  $297.32 \pm 2.53 \mu\text{m}^2$ , and  $177.84 \pm 5.15 \mu\text{m}$ , respectively. The area and perimeter of respiratory bronchioles in group I were  $17.68 \pm 0.49 \mu\text{m}^2$ , and  $26.60 \pm 0.52 \mu\text{m}$  respectively, while those in group II were  $19.28 \pm 0.35 \mu\text{m}^2$ , and  $29.28 \pm 0.35 \mu\text{m}$ , respectively. Mucus was found in the bronchioles and respiratory bronchioles in group II, however, there was no visible mucus observed in group I. In addition, it was also concluded that exposure to low doses of filtered kretek cigarette smoke, 1 cigarette/day for 3 months, increased the expression of the p53 gene in the lungs of rats.

**Conclusion:** The size of bronchioles in rats decreased after being exposed to filtered kretek cigarette smoke 1 stick/day for 3 months, while the size of respiratory bronchioles increased. In addition, exposure to cigarette smoke increased the expression of the p53 gene in the rat lungs.

**Keywords:** Filtered kretek cigarettes smoke, Histometric, Bronchioles, Respiratory bronchioles, p53 gene, Tobacco.

© 2024 The Author(s). Published by Bentham Open.

This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International Public License (CC-BY 4.0), a copy of which is available at: <https://creativecommons.org/licenses/by/4.0/legalcode>. This license permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

\*Address correspondence to this author at the Department of Biology, Faculty of Medicine, Universitas Trisakti, Jl. Kyai Tapa, Kampus B, No.260 Grogol 11440, Jakarta-Indonesia; E-mail: [edyparwanto@trisakti.ac.id](mailto:edyparwanto@trisakti.ac.id)

Cite as: Parwanto E, Tjahyadi D, Sisca S, Amalia H, Hairunisa N, Edy HJ, Oladimeji A, Djebli N. Low Doses of Kretek Cigarette Smoke Altered Rat Lung Histometric, and Overexpression of the p53 Gene. Open Respir Med J. 2024; 18: e18743064285619. <http://dx.doi.org/10.2174/0118743064285619240327055359>



Received: October 29, 2023

Revised: February 26, 2024

Accepted: March 13, 2024

Published: ?? ??, 2024



Send Orders for Reprints to [reprints@benthamscience.net](mailto:reprints@benthamscience.net)

## 1. INTRODUCTION

In Indonesia, many people smoke, especially among young people [1]. Cigarettes can be obtained from the market, but some are made by residents, manually. The main ingredient of cigarettes is tobacco. Cigarettes are one of the major health problems in the world. Even so, many people smoke. It has been reported that cigarettes kill more than 8 million people each year in the world. More than 7 million of those deaths are the result of direct tobacco use, while around 1.2 million are the result of non-smokers being exposed to second-hand smoke [2]. The other reports demonstrated that smoking is a risk factor for various types of diseases, because smokers should stop smoking before the age of 40 years [3]. Especially Indonesia, is one of the countries in Southeast Asia whose population is mostly smokers [4]. Moreover, the number of smokers in Indonesia is very large. In addition, filtered kretek cigarettes are in great demand by most active smokers in Indonesia. This fact shows that most Indonesians smoke kretek.

There are several types of cigarettes in Indonesia, including filtered kretek cigarettes. Generally, filtered kretek cigarettes are made in the factory using machines and are equipped with a filter at one end. In an earlier period, the kretek cigarettes were not filtered at one end. Until now, on the market circulating filtered kretek cigarettes, and non-filtered kretek cigarettes. Filtered kretek cigarettes contain tobacco (as the main ingredient) that is mixed with cloves and sauces [5]. In addition to the added cloves, there are other ingredients to complement the taste of kretek cigarettes, namely sauce.

The scientific name of cultivated tobacco is *Nicotiana tabacum* Linn. Based on a previous report, the Indonesia cigarette industry ( $\pm 80\%$ ) used local tobacco such as Temanggung tobacco, Weleri tobacco, Yogyakarta tobacco, Muntilan Tobacco, Boyolali tobacco, Paiton tobacco, Kasturi tobacco, and Madura tobacco [6]. The dried clove flower buds (*Syzygium aromaticum* (L.) Merr. & L.M.Perry) [7] were used as dough in filtered kretek cigarettes. Filtered kretek cigarette sauce is a unique taste of cigarettes [8]. In Klaten district and its surroundings (Central Java Province, Indonesia), kretek cigarette sauce is known as "woor".

Cigarette smoke contains a complex mixture of chemical compounds. More than 4000 chemical compounds in cigarette smoke have been identified [9]. Cigarette smoke is known to consist of gas (92%) and solid or particulate matter (8%) [10]. Recent research demonstrates that particulate matter emissions from kretek cigarettes can be very high. This is clearly dangerous for both active smokers and non-smokers who are exposed to cigarette smoke [11]. People who smoke, gases and particulate matter in the smoke pour from the mouth to the alveoli. It has been demonstrated that there was deposition of cigarette smoke particles in various locations of the human respiratory tract [12].

Furthermore, it has been demonstrated that cigarette smoke is associated with bronchopulmonary dysplasia

[13]. In addition, cigarette smoke also has free radicals that cause airway disorders, resulting in lung abnormalities. Moreover, cigarette smoke contains particles that damage lung tissue, causing permanent damage called chronic obstructive pulmonary disease (COPD) [14, 15].

Generally, a rat's respiratory system consists of the nose, pharynx, larynx, trachea, primary bronchi, and lungs (bronchioles and alveoli) [16]. The latest research demonstrated that components of rat lung structure are blood vessels, bronchi, terminal bronchi, respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli [17]. In addition, it has been demonstrated about the lower respiratory system of mice (bronchioles, alveolar passages, and alveoli) both qualitatively and quantitatively [18]. A previous study reported that the respiratory systems of rats exposed to gas showed increased tissue elasticity and tissue resistance [19]. A recent study also reported that rats were given conventional cigarette smoke, collagen deposition appearance in the bronchioles, as well as intrabronchiolar mucus [20].

In addition, it is also shown that cigarette smoke affects the anatomical structures of the respiratory system [21]. Another study also showed that smokers have experienced basal cell hyperplasia, mucous cell hyperplasia, and squamous metaplasia [22]. In the application of COPD diagnosis, both qualitative and quantitative assessments are needed [23]. This research is important because bronchioles and respiratory bronchioles are an important part of the lungs associated with the alveoli. We intend to present the quantitative changes in bronchioles, and respiratory bronchioles caused by the effects of filtered kretek cigarette smoke. Quantitative changes in the bronchioles and respiratory bronchioles are important so that the diagnosis related to lung tissue disorders can be determined more objectively. Apart from that, it also needs to be proven whether low doses of kretek cigarette smoke affect gene expression, especially p53. We know that the p53 gene is used as a tumour marker as a key cellular process such as proliferation, apoptosis, and metabolism to suppress tumorigenesis [24]. Therefore, based on the description above, it is necessary to research the histometric of bronchioles, and respiratory bronchioles in Sprague-Dawley rats after treatment with filtered kretek cigarette smoke. Likewise, it is also necessary to prove the dangers of low-dose smoking on over-expression of the p53 gene.

## 2. MATERIALS AND METHODS

### 2.1. Preparation of Filtered Kretek Cigarettes

The type of tobacco used as an ingredient in filtered kretek cigarettes was local tobacco. The types of local tobacco (*Nicotiana tabacum* Linn.) were collected from areas in Manisrenggo District, Klaten Regency, Central Java Province, Indonesia. Cloves (*Syzygium aromaticum* Merr. & L.M. Perry) used in filtered kretek cigarette dough were dried clove flowers, collected from areas in the Logede Village, Karangnongko District, Klaten Regency, Central Java Province, Indonesia. The sauce

known as “woor,” used for filtered kretek cigarettes was a special ingredient that was always used to mix cigarettes manually. These materials were used to make filtered kretek cigarettes manually.

## 2.2. Ethical Clearance

The ethical clearance of this research was obtained from the Research Ethics Commission, Faculty of Medicine, Universitas Trisakti, Jakarta, Indonesia (No. 184/KER-FK/VIII/2018).

## 2.3. Flowchart of Experimental Rat Treatment

The flowchart of experimental rat treatment is presented in Fig. (1).

## 2.4. Animal Care

We used *Rattus norvegicus*, Sprague-Dawley strain. The number of rats per group was calculated using formula  $(n-1) (t-1) \geq 15$ . For each treatment group a minimum of 8 rats were required. Sixty male rats were used in this study. The rats included in this study were 2-3 months old and weighed 150-200 grams. Determination of rat health was carried out after examination by a Veterinary. Rats were caged (individually) in an air-

conditioned room and the room temperature was set to  $22 \pm 3$  °C, humidity  $55 \pm 5\%$ , and artificial fluorescent lamps (12:12 hour light and dark cycle). The rats fed and drank in the libitum according to the standard. There are 2 stages in this research, namely the 1<sup>st</sup> stage in the form of rat acclimatization and the 2<sup>nd</sup> stage was the treatment of giving filtered kretek cigarette smoke to rats.

### 2.4.1. Purchasing Details of the Experimental Rats

Type of animal is rat, Amount 16, Gender is Male Age is 2.5 months Weight 150-200 grams. Systematica (American Fancy Rat and Mouse Association, 2004) - Kingdom - Filum - Sub Filum - Class - Ordo - Sub Ordo - Family - Genus - Species - Strain: Animalia: Chordata: Vertebrata: Mammalia: Rodentia: Myomorpha: Muridae: Rattus: Rattus norvegicus: Sprague Dawley

## 2.5. Treatment on Sprague-dawley Rats

Trial of filtered kretek cigarette smoke on rats was carried out at The Veterinary Medical Teaching Hospital (Rumah Sakit Hewan Pendidikan=RSHP), Faculty of Veterinary Medicine of Bogor Agricultural University. This research was conducted between November 2019 - April 2020.

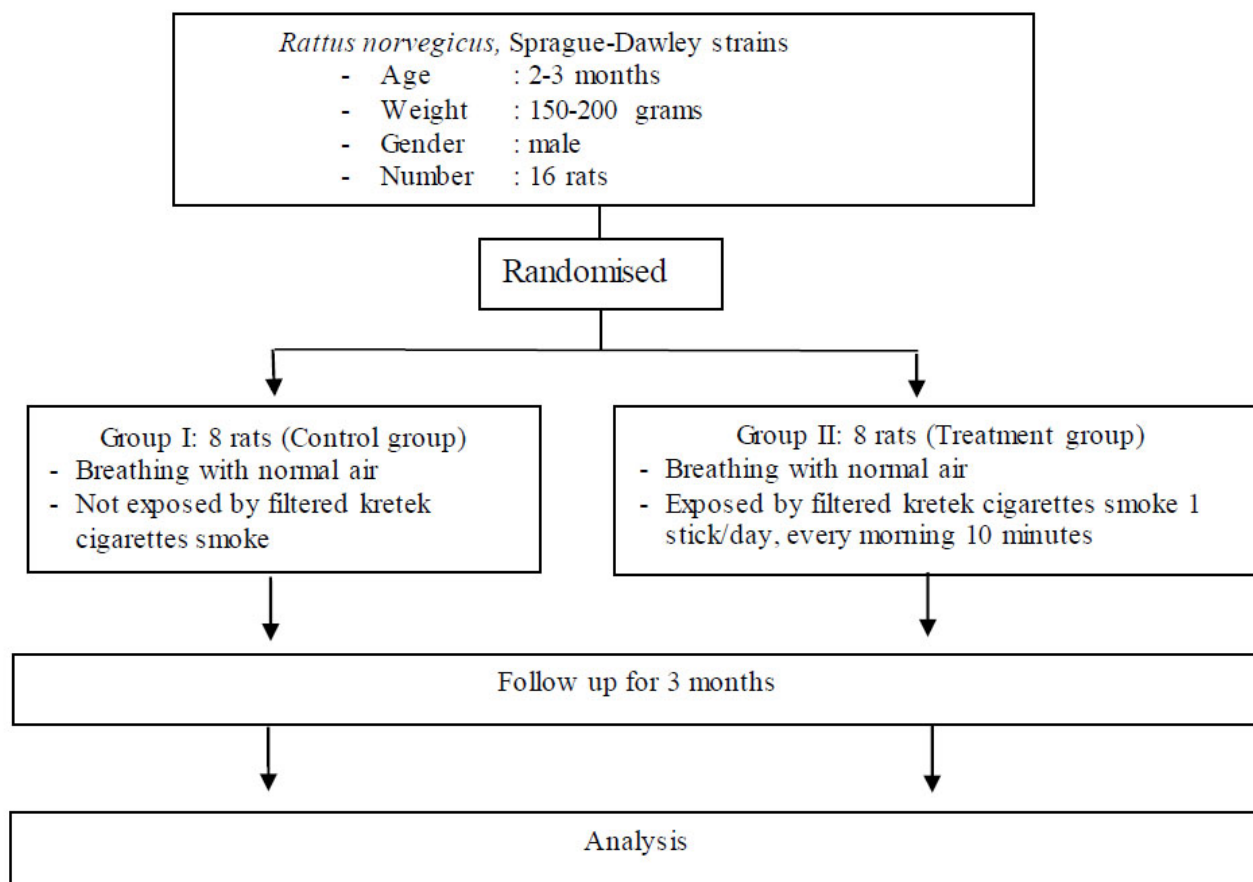
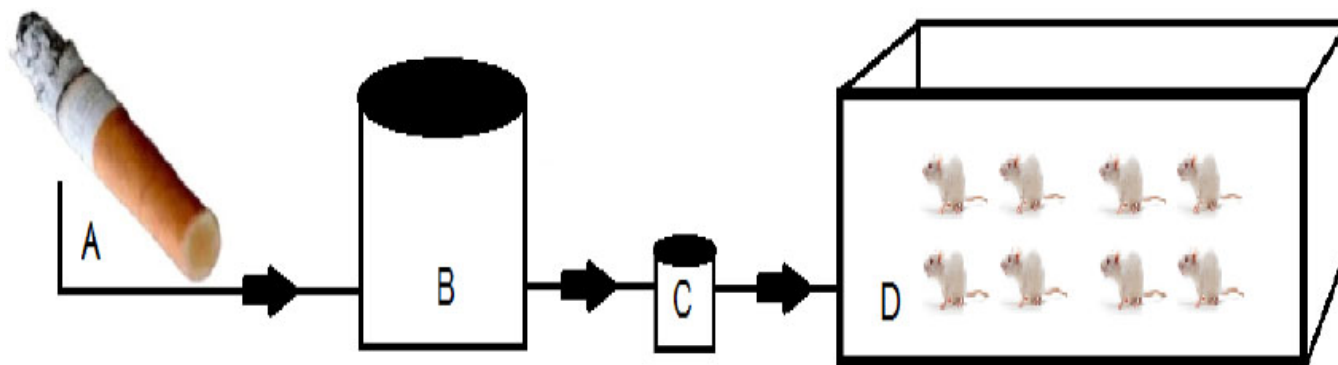


Fig. (1). Flowchart of experimental rat treatment.



**Fig. (2).** Schematic diagram of filtered kretek cigarettes smoke exposure to a groups of Sprague-Dawley rats. **A.** Filtered kretek cigarettes burned. **B.** Tube to accommodate filtered kretek cigarettes smoke. **C.** Electric pump. **D.** Glass box for rats treatment with length 40 cm, width 30 cm, and height 20 cm).

Acclimatization of rats was carried out for 2 weeks, then continued with treatment according to groups. Rats were divided into 2 groups with randomization. Group I was the rat group that was breathing normal air, and was not exposed to filtered kretek cigarette smoke (as a control). Group II, namely the rat group exposed to filtered kretek cigarettes smoke 1 stick/day/group (Fig. 2). The treatment duration for the two groups of rats was 3 months. After the treatment, the rats were sacrificed, and then their respiratory organs were collected from the trachea to the lungs to make histology slides.

**2.6. Measurement of Bronchioles and Respiratory Bronchioles**

The tissue was immersed in a neutral buffer solution of 10% formalin for 24 hours at room temperature. The tissue was cut to a size of 1x1x1 cm, and then put in a tissue cassette. The tissue was then transferred to alcohol dehydration with alcohol concentrations of 70%, 80%, 90%, and 96%, respectively. The dehydration time was 2 hours for each alcohol concentration. The next stage was clearing, after which, the tissue was ready to be inserted into the paraffin block. Furthermore, embedding and blocking were carried out. The tissue in the paraffin block was cut using a microtome with a thickness of 4-5 μ, and

then stained with hematoxylin and eosin.

Image documentation using Optilab Advance Plus and Image Raster 3 by PT MICONOS, Daerah Istimewa Yogyakarta, Indonesia; available at <https://miconos.ac.id/new/support/download>. Optilab Advance Plus and Image Raster 3 programs were used to analyze rat respiratory system histometrics (bronchioles and respiratory bronchioles) by three observers. Data for all groups were expressed as mean± standard deviation. Analyzing data between groups using an independent sample t-test. The difference between groups was significant if the test results showed P<0.05.

**2.7. Expression of p53 Gene in Rat Lung**

Measurement of p53 gene expression was carried out using real-time polymerase chain reaction (RT PCR). Lung tissue samples of Sprague Dawley rats were isolated for mRNA, and then the solution was put into a 1.5 mL tube, stored at -80 °C. The reagents for measuring mRNA levels were Bioline SensiFast Sybr Lo-ROX One-step kit, Zymo Research Quick-RNATM MiniPrep Plus, and Geneaid Micropestle. The p53 rat target gene used the sense primer (5' to 3') AGGCCTTGGAAGTCAAGGAT, while the antisense primer (5' to 3') TGAGTCAGGCCCTTCTGTCT, with a size of 140 bp. The glyceraldehyde-3-phosphate

**Table 1. Comparison of the effect of filtered kretek cigarette smoke on the Sprague Dawley rat lung.**

Sprague Dawley Rat Lung	Treatment Groups		p value
	Group I (n=8)	Group II (n=8)	
<b>-Bronchioles:</b>			
Length (μm)	40.42 ± 1.58	30.77 ± 0.78	0.000
Width (μm)	14.82 ± 0.41	9.28 ± 0.40	0.000
Area (μm <sup>2</sup> )	494.61 ± 5.62	297.32 ± 2.53	0.000
Perimeter (μm)	233.87 ± 4.51	177.84 ± 5.15	0.000
<b>-Respiratory bronchioles</b>			
Area (μm <sup>2</sup> )	17.68 ± 0.49	19.28 ± 0.38	0.000
Perimeter (μm)	26.60 ± 0.52	29.28 ± 0.35	0.000
<b>-Expression of p53 gene</b>	1 ± 0.00	3.02 ± 0.69	0.000

**Abbreviations:** Group I, rats breathe using ordinary air without exposure to filtered kretek cigarette smoke. Group II, the group of rats exposed to filtered kretek cigarette smoke 1 stick/day for 3 months of treatment.

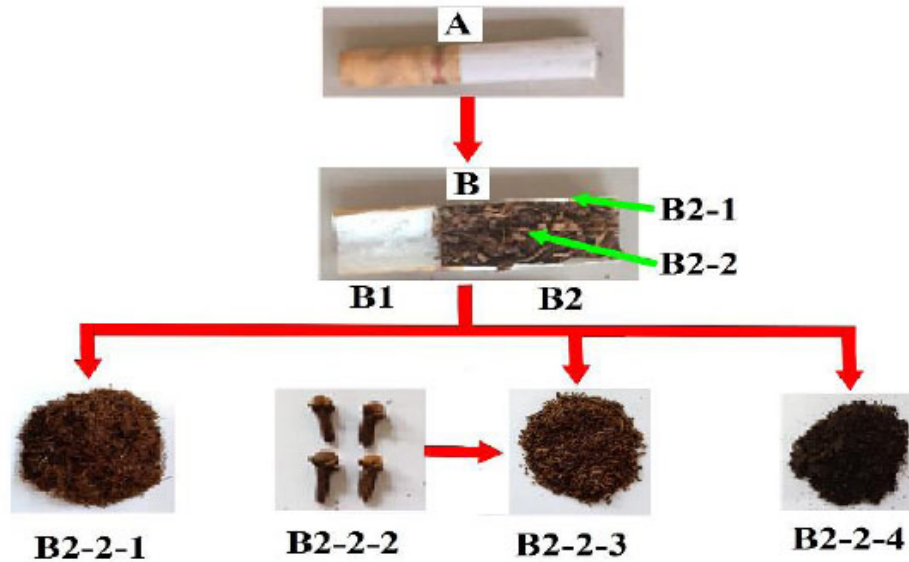
dehydrogenase (GAPDH) gene was used as an internal control. The sense primer of GAPDH gene was (5' to 3') CCAGGTGGTCTCCTCTGACTTCTC, while the antisense primer was (5' to 3') ATACCAGGAAATGAGCTTGACA, with a size of 147 bp [25]. RT PCR analysis using SYBR Green Master Mix (Bio-rad) in the Bio-rad CFX96 RT PCR system. Ct p53 gene was normalized with Ct gene GAPDH. The calculation was based on the  $2^{-\Delta\Delta Ct}$  method (Livak method)

[26], as demonstrated in Table 1.

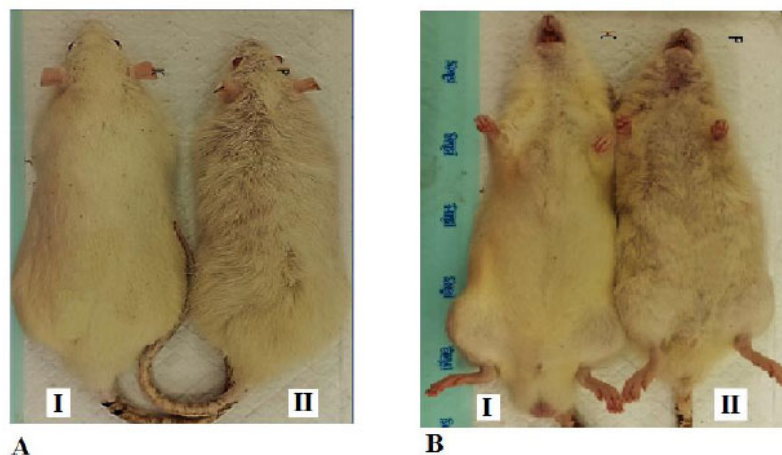
### 3. RESULTS

#### 3.1. Filtered Kretek Cigarettes

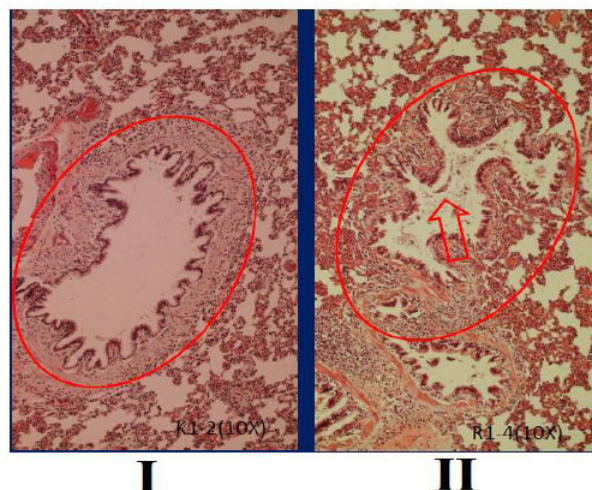
The constituent materials of filtered kretek cigarettes are presented in Fig. (3). Filtered kretek cigarettes consist of filters and kretek dough. Tobacco is the main ingredient of kretek cigarettes, which is mixed with cloves and sauce.



**Fig. (3).** Composition of filtered kretek cigarettes. (A). The appearance of filtered kretek cigarettes. (B). Filtered kretek cigarettes split lengthwise so that it appears the inside which consists of the filter (B1) and kretek section (B2). B2-1. Wrapping paper for filtered kretek cigarettes dough. B2-2-1. Tobacco as the main constituent of filtered kretek cigarettes dough. B2-2-2. Dried clove flower. B2-2-3. Chopped dried clove flower. B2-2-4. Kretek sauce.



**Fig. (4).** Hair of the Sprague-Dawley rats. **A.** Hair appearance of the back body. **B.** Hair appearance of the abdomen. I. Group I, rats breathe using ordinary air without exposure to filtered kretek cigarette smoke. II. Group II, the group of rats exposed to filtered kretek cigarette smoke 1 stick/day for 3 months of treatment.



**Fig. (5).** Bronchioles histological of Sprague-Dawley rats, stained with hematoxylin and eosin, objective10x. I. Group I, rats breathe using ordinary air without exposure to filtered kretek cigarette smoke. II. Group II, the group of rats exposed to filtered kretek cigarette smoke 1 stick/day for 3 months of treatment. The red arrows showed mucus.

### 3.2. Hair Appearance

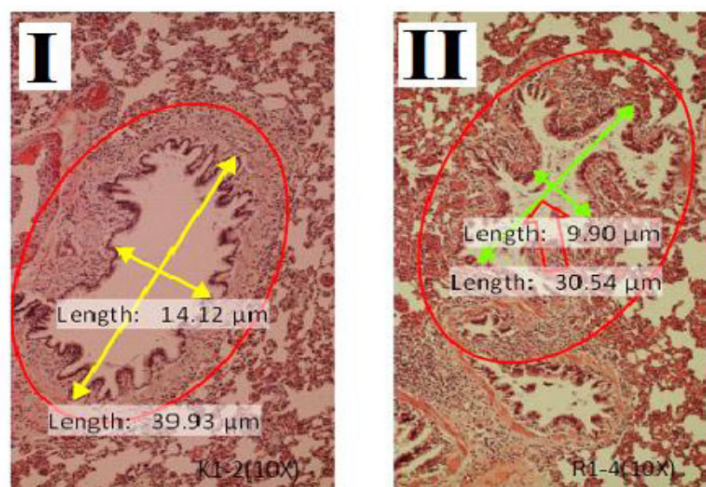
The hair appearance of rats between group I and group II is presented in Fig. (4). The hair on the back and abdomen of rats in group I looks white, clean, and dense, while in group II, it looks dull yellow, less clean, and sparse.

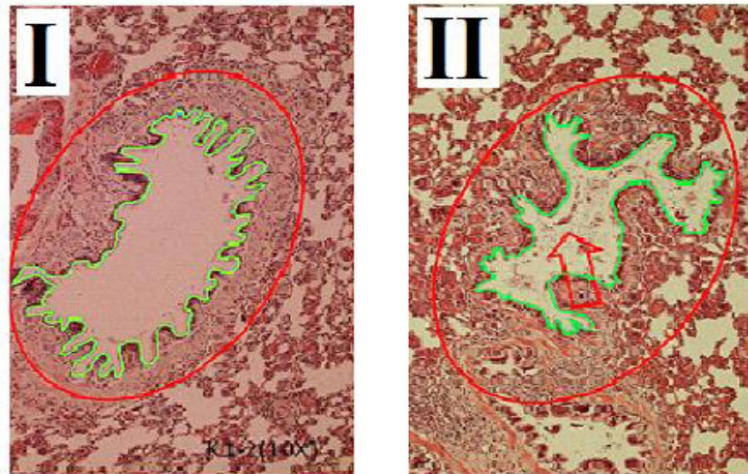
### 3.3. Bronchioles Histometric

Histological of bronchioles in Sprague-Dawley rats between group I and group II is presented in Fig. (5). Treatment of filtered kretek cigarettes smoke 1 stick/day for 3 months causes mucus in the bronchioles space of rats in group II, while in group I, it is not found. In addition, the size of the bronchioles space also appears to be shrinking.

A comparison of bronchioles histometric in Sprague-

Dawley rats between group I and group II is presented in Fig. (6). Based on Fig. (6), seen that bronchioles length, width, area, and perimeter in group I are different compared to group II ( $P=0.000$ ). Bronchioles histometric of rats in group II < I ( $P=0.000$ ). In this study, the group of rats exposed to filtered kretek cigarette smoke had changes in bronchioles histometric, namely the decrease in length, width, area, and perimeter. The bronchioles length of rats in group II decreased by 24.14% compared to group I, while the bronchioles width of rats in group II decreased by 37.38% compared to group I. The bronchioles area of rats in group II decreased by 39.89% compared to group I, while the bronchioles perimeter of rats in group II decreased by 23.96% compared to group I. Rats in group II exert a negative response against filtered kretek cigarette smoke in the form of a decrease in the size of bronchioles histometric.





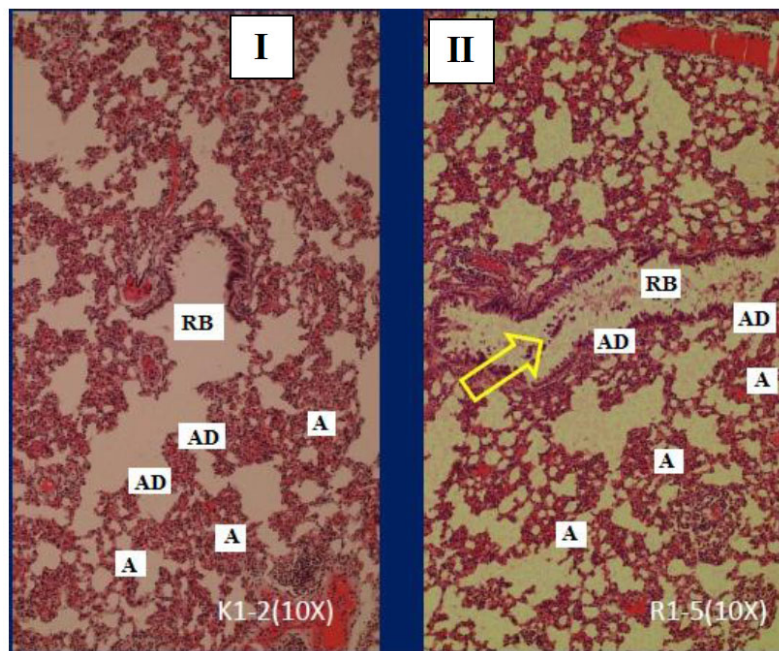
B

**Fig. (6).** Comparison of bronchioles histometric in Sprague-Dawley rats between group I compared to group II, staining with hematoxylin and eosin, objective10x. (A). Length and width of the bronchioles. (B). Area and perimeter of the bronchioles. I. Group I, rats breathe using ordinary air without exposure to filtered kretek cigarette smoke. II. Group II, the group of rats exposed to filtered kretek cigarette smoke 1 stick/day for 3 months of treatment. The red arrows showed mucus.

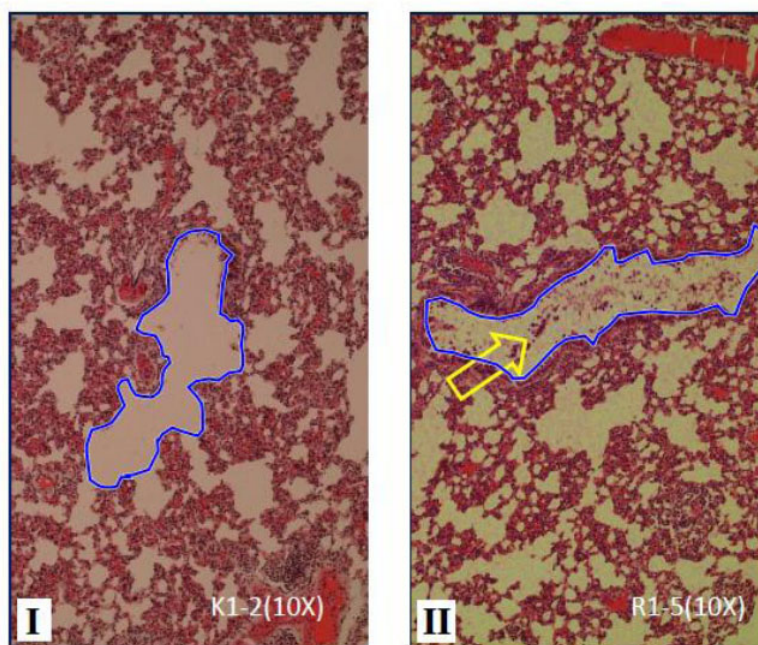
### 3.4. Respiratory Bronchioles Histometric

The histological of respiratory bronchioles in Sprague-Dawley rats between group I and group II is presented in

Fig. (7). Based on Fig. (7) treatment of filtered kretek cigarettes smoke 1 stick/day for 3 months causes mucus in the respiratory bronchioles space of Sprague-Dawley rats in group II, while in group I is not found.



**Fig. (7).** The histological of respiratory bronchioles in Sprague-Dawley rats between group I compared to group II, stained with haematoxylin and eosin, objective 10x. The yellow arrows showed mucus. RB= respiratory bronchioles, AD= alveolar ducts, A=alveoli. I. Group I, rats breathe using ordinary air without exposure to filtered kretek cigarette smoke. Group II, the group of rats exposed to filtered kretek cigarette smoke 1 stick/day for 3 months of treatment.



**Fig. (8).** Comparison of area and perimeter of respiratory bronchioles in Sprague-Dawley rats between group I compared to group II, stained with haematoxylin and eosin, objective 10x. Respiratory bronchioles of rats in group II showed mucus (yellow arrow), while in the group I mucus was not found. I. Group I, rats breathe using ordinary air without exposure to filtered kretek cigarette smoke. II. Group II, the group of rats exposed to filtered kretek cigarette smoke 1 stick/day for 3 months of treatment.

The area and the perimeter of respiratory bronchioles in the Sprague-Dawley rats between group I and group II is presented in Fig. (8). Fig. (8) demonstrated that the area and the perimeter of respiratory bronchioles of rats in group I < group II ( $P=0.000$ ). In this study, the group of rats exposed to filtered kretek cigarette smoke had changes in respiratory bronchioles histometric, namely the increase in area and perimeter. The area of respiratory bronchioles of rats in group II increased by 9.05% compared to group I, while the perimeter of respiratory bronchioles of rats in group II increased by 10.075% compared to group I. Rats in group II had a positive response against filtered kretek cigarette smoke in the form of an increase in the area and perimeter of respiratory bronchioles.

### 3.5. Expression of p53 Gene

Optimization of RT PCR for the determination of mRNA levels for p53 is presented in Fig. (9). Based on the measurement data, it was demonstrated that the treatment of rats exposed to filtered kretek cigarette smoke 1 cigarette/day for 3 months influenced p53 gene expression. There was an increase in p53 gene expression in rats exposed to filtered kretek cigarette smoke. It was proven that the mRNA levels of group II were higher (upregulation) than group I ( $P=0.000$ ).

## 4. DISCUSSION

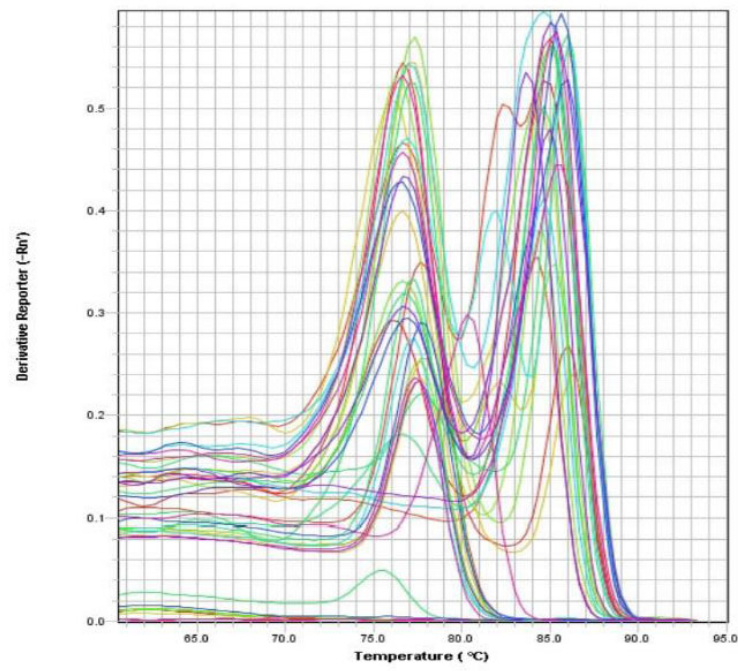
The color of the filter on filtered kretek cigarettes before burning was different from after burning. Before

the filtered kretek cigarettes were burned, the filter was white, while after they were burned, the filter had a burlywood color. It has been demonstrated that the function of the filter at the end of the filtered kretek cigarettes is to reduce tar and nicotine levels that enter the respiratory tract. The color change of the burlywood filter in this study indicated the presence of tar and nicotine from filtered kretek cigarettes. This fact is in line with previous research [27].

Filtered kretek cigarettes that were burning produced cigarette smoke and ash. Cigarette smoke was inhaled by rats, while ash of filtered kretek cigarettes was disposed of. Most of the filtered kretek cigarette smoke entered the respiratory system of rats. Filtered kretek cigarette smoke is one of the incomplete combustion products of tobacco, cloves, and sauce. Cigarette smoke includes main cigarette smoke and side cigarette smoke. Main cigarette smoke is cigarette smoke that was inhaled by rats as a model of active smokers. Side cigarette smoke is cigarette smoke that was in the environment around rats. In this study, filtered kretek cigarettes burned, and a pump mainly inhaled cigarette smoke and then channeled into the smoking box. This is similar to the smoking process that is generally carried out by smokers. The results of this study clearly show that there are differences in the color of the filters before and after the filtered kretek cigarettes burned. This fact clarifies the filter function of the filtered kretek cigarettes, which is to filter particles in cigarette smoke that are inhaled by smokers. Therefore, the filter on filtered kretek cigarettes can reduce the

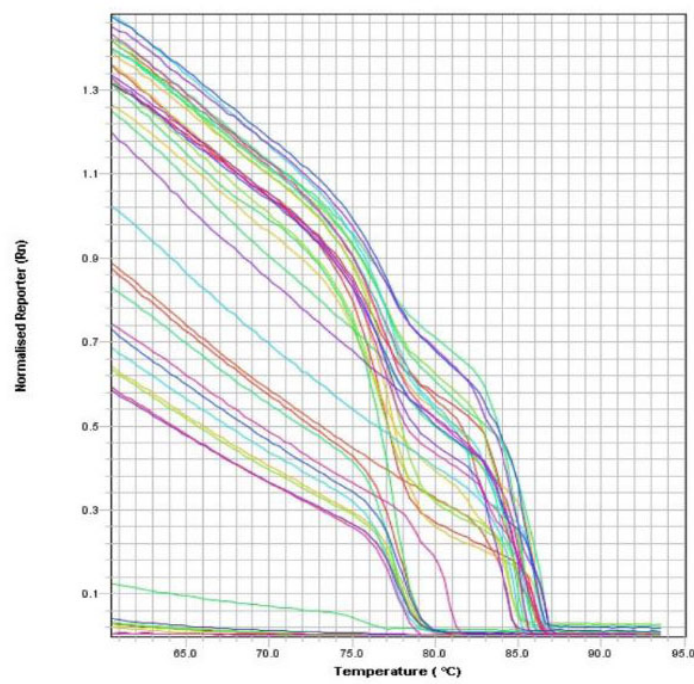


Melt Curve (Derivative Reporter)



A.

Melt Curve (Normalized Reporter)



B.

Fig. (9). Optimization of RT PCR for p53 gene expression. (A). Melting curve (derivative curve); (B). Melting curve (normalized reporter).

content of substances inhaled when smoking. Substances in cigarette smoke that have been shown to be high in content include nicotine, various metals [28], free radicals [29], oxidants and antioxidants [30]. Even though there is a filter, cigarette smoke in this study was proven to affect the hair appearance of rats, which worsened hair appearance.

Exposure to filtered kretek cigarette smoke in this study caused the hair of rats to change from pure white to dull yellow. The hair discoloration occurred throughout the rat's body. The results in this study were in accordance with the results of the report which stated that the smoker's mustache hair turned yellow [31]. Filtered kretek cigarette smoke in this study mostly comes from tobacco leaves, so it has nicotine. Nicotine in the filtered kretek cigarette smoke enters the body and then causes discoloration, cleanliness, and density of hair in the rats. This fact is in accordance with the results of earlier studies, which show that the nicotine content of hair is used as a biomarker to assess chronic exposure to environmental tobacco smoke [32]. Nicotine in cigarette smoke by inhalation enters the body and penetrates cell membranes to the systemic circulation system. It is further stated that nicotine is the main constituent of tobacco. Nicotine is not carcinogenic, but it triggers many carcinogens in tobacco [33]. Previous research has demonstrated that nicotine levels in the hair of smokers are much higher compared to non-smokers [34]. In addition to nicotine content, cigarette smoke also causes oxidative stress that affects the condition of the hair and, among others, affects the hair fiber [35]. In addition to affecting hair color, cigarette smoke also affects histometrics in the lungs of rats, including bronchioles and respiratory bronchioles.

The reduction in length, width, area, and perimeter of bronchioles in rats in this study responded to unwanted cigarette smoke exposure because, in normal conditions, the respiratory air does not contain cigarette smoke. This occurs as a defense mechanism for reducing the volume of cigarette smoke exposure. Reduction of bronchioles histometric reduces exposure to filtered kretek cigarette smoke, next to reducing the volume of cigarette smoke until it reaches the alveolar sacs (alveolar saccules). This situation allows obstruction of airflow that cannot flow back completely. This fact is consistent with earlier studies showing that the bronchioles are the site of airway obstruction. In addition to bronchioles abnormalities due to cigarette smoke, it was found that cigarette smoke also causes COPD [36]. Based on the facts obtained, we agree that pulmonary histometrics is useful for quantitatively measuring the tissues that make up the organs in the respiratory system. Moreover, it has been demonstrated that exposure to filtered kretek cigarettes at a dose of 1 cigarette/day or 2 cigarettes/day for 30 days, causes bronchial mucosal hyperplasia and bronchoconstriction in male Sprague Dawley rats [37]. Apart from that, it has also been demonstrated that filtered kretek cigarette smoke not only impacts the respiratory organs but also impacts Purkinje cells and pyramidal cells in Sprague Dawley rats [38].

In addition, the facts in this study, in accordance with the results of other studies which, indicated that structural abnormalities and blockage by mucus can cause destabilization of the airways in the bronchioles [39].

Previous studies have shown that morphometric measurements of rat lungs have been carried out [17]. In addition to the morphometric analysis of the rat lungs, pulmonary histometrics have also been shown. The results of the study indicated that the diameter of the respiratory bronchioles, alveolar ducts, and alveoli were  $24.93 \pm 1.27 \mu\text{m}$ ,  $21.14 \pm 0.66 \mu\text{m}$ , and  $12.95 \pm 0.21 \mu\text{m}$ , respectively [18]. Need to note that it is not only cigarette smoke that causes chronic obstruction of the bronchioles. It has been shown that chronic obstruction of the bronchioles can also be caused by cell degeneration in the bronchioles [40]. Therefore, it is still necessary to prove the effect of cigarette smoke on cell death in the bronchioles.

The effect of filtered kretek cigarette smoke in this study caused the area and perimeter of respiratory bronchioles in group II of rats to be greater compared to group I as a control. The effect of filtered kretek smoke in this study narrowed the bronchioles space (Fig. 6) but widened the respiratory bronchioles space (Fig. 8). The increased respiratory bronchioles space may be due to the pressure of filtered kretek cigarette smoke, such as the alveolar sac. The changes in histometric in the respiratory bronchioles in this study are in line with the other studies showing significant changes in the respiratory bronchioles of rats after treatment with hypoxia and melatonin [41].

Our results showed that mucus appeared in the bronchioles and respiratory bronchioles of rats. Filtered kretek cigarette smoke 1 cigarette/day for 3 months caused mucus in the respiratory bronchioles space in group II, while in group I (control), this mucus was not found (Figs. 5-8). Of note, apart from in the bronchioles and respiratory bronchioles, mucus is also found in the saccus alveolar space (data not shown). The mucus is a complex mixture of mucins. Mucins are produced by airway epithelial cells and submucosal glands [42]. As a result of mucus that has accumulated in the bronchioles and respiratory bronchioles, airflow in the lungs is limited, thus resulting in lung disorders. In addition, demonstrated that inflammatory cells produce excess mucus which reduces lung function [43].

Previous studies demonstrated that cigarette smoke causes oxidative stress due to an imbalance between oxidants and antioxidants. Furthermore, cigarette smoke also induces epithelial cells for mucus hypersecretion, increasing proinflammatory cytokines and chemokines. Cigarette smoke has also been shown to increase macrophages and neutrophils and disrupt the balance of lymphocytes [30]. Cigarette smoke causes mucus hypersecretion and COPD. In addition, exposure to cigarette smoke can worsen COPD [44]. The results of other studies have also demonstrated that smoking at a young age is a dominant factor in the cause of COPD [45]. Based on the results of this study and several other studies, it is clear that cigarette smoke has a complex effect on lung tissue [46]. Moreover, cigarette smoke induces epigenetic changes in lung tissue [47], lung cancer [48, 49], oral disease, myocardial infarction, oral cancer, chronic health disease, cardiovascular disease, and asthma [50].

The Ct of the p53 gene for group I is 1, while the Ct p53 gene for group II shows a multiple of 1, so it experiences upregulation [51]. Of the 8 samples in this study, all showed up-regulation, and none showed downregulation. Rats exposed to filtered kretek cigarette smoke showed increased lung mRNA levels of the p53 gene. The occurrence of up regulation of the p53 gene in mice exposed to low doses of filtered kretek cigarette smoke in this study is in accordance with the statement relating to the processes of proliferation, apoptosis, and metabolism to suppress tumorigenesis [24]. It was further stated that the p53 gene plays an important role in responding to various types of stress cells [52]. The results of this study can be used as a basis for further research on tobacco consumption (cigarettes) with the occurrence of lung cancer. This is in accordance with research results, which demonstrate that tobacco use and variations in the p53 gene increase the risk of oral cancer [53, 54].

## 5. LIMITATIONS

The present study has limitations; among others, we did not measure the levels of chemical compounds in filtered kretek cigarette smoke. In this study, we have not determined damage to the bronchioles and respiratory bronchioles based on **histometrics due** to exposure to filtered kretek cigarette smoke. In addition, we have not performed cytometrics on bronchioles and respiratory bronchioles as we did before with human cervical cells [54, 55]. We hope that in the next opportunity, we can demonstrate the relationship between the levels of chemical compounds in filtered Kretek cigarette smoke with the level of the bronchioles and respiratory bronchioles damage based on cytometric and histometric.

## CONCLUSION

Based on the results obtained, we concluded that Sprague-Dawley rats in group II were exposed to 1 stick/day of filtered kretek cigarette smoke for 3 months and showed dull yellow hair on the back and abdomen, less clean, and less frequent compared **to the group** I (control). The size of bronchioles of Sprague-Dawley rats decreased after being exposed to filtered kretek cigarettes smoke 1 stick/day for 3 months, while the size of **respiratory bronchioles** increased. Mucus is found in the bronchioles and **respiratory bronchioles** of Sprague-Dawley rats. Mucus was found in the bronchioles, and **respiratory bronchioles** in the group were exposed to 1 stick/day of filtered kretek cigarette smoke for 3 months, while in the control group, there was no visible mucus. It is clear that the quantitative changes in bronchioles and respiratory bronchioles are caused by the effects of filtered kretek cigarettes smoke. Quantitative changes in the bronchioles and respiratory bronchioles are important so that the diagnosis related to lung tissue disorders can be determined more objectively. In addition, it was also shown that exposure to low doses of filtered kretek cigarette smoke, 1 cigarette/day for 3 months, increased the expression of the p53 gene (**upregulation**) in the rat lungs.

## SIGNIFICANCE STATEMENT

This study discovered that exposure to low-dose filtered kretek cigarette **smoke changed the histometric of bronchioles and respiratory bronchioles** in the Sprague-Dawley rats model. In addition, exposure to low-dose filtered kretek cigarette smoke also showed an increase in p53 gene expression in rat lungs.

## LIST OF ABBREVIATIONS

COPD = Chronic Obstructive Pulmonary Disease

RT PCR = Real-time Polymerase Chain Reaction

mRNA = Messenger ribonucleic acid

GAPDH = Glyceraldehyde-3-phosphate dehydrogenase

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The ethical clearance of this research was obtained from the Research Ethics Commission, Faculty of Medicine, Universitas Trisakti, Jakarta, Indonesia (No. 184/KER-FK/VIII/2018).

## HUMAN AND ANIMAL RIGHTS

No humans were used for studies that are the basis of this study. This study adheres to internationally accepted standards for animal research, following the 3Rs principle. The ARRIVE guidelines were employed for reporting experiments involving live animals, promoting ethical research practices.

## CONSENT FOR PUBLICATION

Not applicable.

## AVAILABILITY OF DATA AND MATERIAL

All the data and supporting information is provided within the article.

## FUNDING

This work is supported by the Faculty of Medicine, Universitas Trisakti, Indonesia. This research funding is in the budget year 2019-2020.

## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

## ACKNOWLEDGEMENTS

Declared none.

## REFERENCES

- [1] Kasri R, Ahsan A, Wiyono NH, Jacinda A, Kusuma D. New evidence of illicit cigarette consumption and government revenue loss in Indonesia. *Tob Induc Dis* 2021; 19: 1-8. <http://dx.doi.org/10.18332/tid/142778> PMID: 34782828
- [2] WHO. Tobacco. 2020. Available From: <https://www.who.int/news-room/fact-sheets/detail/tobacco>
- [3] Jha P. The hazards of smoking and the benefits of cessation: A critical summation of the epidemiological evidence in high-income countries. *eLife* 2020; 9: e49979. <http://dx.doi.org/10.7554/eLife.49979> PMID: 32207405

- [4] SEATCA (Southeast Asia Tobacco Control Alliance). Tobacco Control Atlas ASEAN Region. 2018. Available From: [https://seatca.org/dmdocuments/TobaccoControlAtlas\\_ASEANRegion\\_4thEd\\_Dec2019.pdf](https://seatca.org/dmdocuments/TobaccoControlAtlas_ASEANRegion_4thEd_Dec2019.pdf)
- [5] Palipudi K, Mbulo L, Kosen S, et al. A cross sectional study of kretek smoking in Indonesia as a Major risk to public health. Asian Pac J Cancer Prev 2015; 16(16): 6883-8. <http://dx.doi.org/10.7314/APJCP.2015.16.16.6883> PMID: 26514461
- [6] The Indonesian Sweetener and Fiber Crops Research Institute (ISFCRI). Tobacco 2015. Available From: <http://balittas.litbang.pertanian.go.id/index.php/en/komoditas/tobacco>
- [7] ITIS (Integrated Taxonomi Information System). *Syzygium aromaticum* (L.) Merr. & L.M. Perry. Taxonomic Serial No.: 506167. 2020. Available From: <https://www.itis.gov/servlet/SingleRpt/SingleRpt/>
- [8] Arnez M. Tobacco and Kretek: Indonesian drugs in historical change. 2009. Available From: <https://nbn-resolving.org/urn:nbn:de:0168-ssoar-362761>
- [9] Lin J, Taggart M, Borthwick L, et al. Acute cigarette smoke or extract exposure rapidly activates TRPA1-mediated calcium influx in primary human airway smooth muscle cells. Sci Rep 2021; 11(1): 9643. <http://dx.doi.org/10.1038/s41598-021-89051-4> PMID: 33953304
- [10] Amorós-Pérez A, Cano-Casanova L, Román-Martínez MC, Lillo-Ródenas MA. Comparison of particulate matter emission and soluble matter collected from combustion cigarettes and heated tobacco products using a setup designed to simulate puffing regimes. Chem Eng J Adv 2021; 8(100144): 100144. <http://dx.doi.org/10.1016/j.cej.2021.100144>
- [11] Braun M, Fromm EL, Gerber A, Klingelhöfer D, Müller R, Gronenberg DA. Particulate matter emissions of four types of one cigarette brand with and without additives: A laser spectrometric particulate matter analysis of secondhand smoke. BMJ Open 2019; 9(1): e024400. <http://dx.doi.org/10.1136/bmjopen-2018-024400> PMID: 30782733
- [12] Paul AR, Khan F, Jain A, Saha SC. Deposition of smoke particles in human airways with realistic waveform. Atmosphere (Basel) 2021; 12(7): 912. <http://dx.doi.org/10.3390/atmos12070912>
- [13] González-Luis GE, van Westering-Kroon E, Villamor-Martínez E, et al. Tobacco Smoking during pregnancy is associated with increased risk of Moderate/Severe Bronchopulmonary Dysplasia: A systematic review and meta-analysis. Front Pediatr 2020; 8(160): 160. <http://dx.doi.org/10.3389/fped.2020.00160> PMID: 32411634
- [14] WHO. WHO highlights huge scale of tobacco-related lung disease deaths. 2019. Available From: <https://www.who.int/news/item/29-05-2019-who-highlights-huge-scale-of-tobacco-related-lung-disease-deaths>
- [15] Carvalho JL, Miranda M, Fialho AK. Oral feeding with probiotic *Lactobacillus rhamnosus* attenuates cigarette smoke-induced COPD in C57Bl/6 mice: Relevance to inflammatory markers in human bronchial epithelial cells. PLoS One 2020; 15(4): e0225560. <http://dx.doi.org/10.1371/journal.pone.0225560>
- [16] Shraideh ZA, Najjar HN. Histological changes in tissues of trachea and lung alveoli of albino rats exposed to the smoke of two types of Narghile Tobacco Products. 2011. Available From: <http://ijbs.hu.edu.jo/FILES/v4n4/Paper%20Number%205.pdf>
- [17] Maina JN, Igbokwe CO. Comparative morphometric analysis of lungs of the semifossorial giant pouched rat (*Cricetomys gambianus*) and the subterranean Nigerian mole rat (*Cryptomys foxi*). Sci Rep 2020; 10(1): 5244. <http://dx.doi.org/10.1038/s41598-020-61873-8> PMID: 32251351
- [18] Ibe CS, Onyeansi BI, Salami SO, Nzalak JO. Microscopic anatomy of the lower respiratory system of the African Giant Pouched Rat (*Cricetomys gambianus*, Waterhouse 1840). Int J Morphol 2011; 29(1): 27-33. <http://dx.doi.org/10.4067/S0717-95022011000100004>
- [19] Serra DS, De Brito KBP, Oliveira KL. Respiratory system of rats exposed to pollutants arising out of heating residual glycerol. J Fundament Renewable Energy Appl 2017; 7(1): 1-4. <http://dx.doi.org/10.4172/2090-4541.1000220>
- [20] Wawryk-Gawda E, Chylińska-Wrzos P, Zarobkiewicz M, Chłapek K, Jodłowska-Jędrych B. Lung histomorphological alterations in rats exposed to cigarette smoke and electronic cigarette vapour. Exp Ther Med 2020; 19(4): 2826-32. <http://dx.doi.org/10.3892/etm.2020.8530> PMID: 32256766
- [21] Kim V, Criner GJ. Chronic bronchitis and chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2013; 187(3): 228-37. <http://dx.doi.org/10.1164/rccm.201210-1843CI> PMID: 23204254
- [22] Zuo WL, Yang J, Gomi K, Chao I, Crystal RG, Shaykhiyev R. EGF-amphiregulin interplay in airway stem/progenitor cells links the pathogenesis of smoking-induced lesions in the human airway epithelium. Stem Cells 2017; 35(3): 824-37. <http://dx.doi.org/10.1002/stem.2512> PMID: 27709733
- [23] Chauhan NS, Sood D, Takkar P, Dhadwal DS, Kapila R. Quantitative assessment of airway and parenchymal components of chronic obstructive pulmonary disease using thin-section helical computed tomography. Pol J Radiol 2019; 84: 54-60. <http://dx.doi.org/10.5114/pjr.2019.82737> PMID: 31019595
- [24] Fischer M. Census and evaluation of p53 target genes. Oncogene 2017; 36(28): 3943-56. <http://dx.doi.org/10.1038/onc.2016.502> PMID: 28288132
- [25] Petre CE, Sin SH, Dittmer DP. Functional p53 signaling in Kaposi's sarcoma-associated herpesvirus lymphomas: Implications for therapy. J Virol 2007; 81(4): 1912-22. <http://dx.doi.org/10.1128/JVI.01757-06> PMID: 17121789
- [26] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25(4): 402-8. <http://dx.doi.org/10.1006/meth.2001.1262> PMID: 11846609
- [27] Sarikaya Y, Kahya ND, Pekdemir AD, Önal M. Removing tar and nicotine from mainstream cigarette smoke using sepiolite-modified filter tips. Clay Miner 2020; 55(4): 375-82. <http://dx.doi.org/10.1180/clm.2021.7>
- [28] Wallin C, Sholtz SB, Österlund N, et al. Alzheimer's disease and cigarette smoke components: Effects of nicotine, PAHs, and Cd(II), Cr(III), Pb(II), Pb(IV) ions on amyloid- $\beta$  peptide aggregation. Sci Rep 2017; 7(1): 14423. <http://dx.doi.org/10.1038/s41598-017-13759-5> PMID: 29089568
- [29] Anggraeni NKN, Djoko DJHS, Juswono UP. Identification of free radical in main stream cigarette smoke in cigarette with clove mix (Kretek) and cigarette without clove mix (White). Buletin Fisika 2020; 212(73): 81.
- [30] Strzelak A, Ratajczak A, Adamiec A, Feleszko W. Tobacco smoke induces and alters immune responses in the lung triggering inflammation, allergy, asthma and other lung diseases: A mechanistic review. Int J Environ Res Public Health 2018; 15(5): 1033. <http://dx.doi.org/10.3390/ijerph15051033> PMID: 29883409
- [31] Laborada J, Cohen PR. Smoker's mustache revisited: Upper lip hair yellow discoloration associated with tobacco. Cureus 2021; 13(10): e18988. <http://dx.doi.org/10.7759/cureus.18988> PMID: 34820243
- [32] Nadhiroh SR, Djokosujono K, Utari DM, Hasugian AR. Questionnaire-based environmental tobacco smoke exposure and hair nicotine levels in 6-month-old infants: A validation study in Indonesia. Glob Pediatr Health 2020; 7: 2333794X2096928. <http://dx.doi.org/10.1177/2333794X20969287> PMID: 33195749
- [33] Murphy SE. Biochemistry of nicotine metabolism and its relevance to lung cancer. J Biol Chem 2021; 296(100722): 100722. <http://dx.doi.org/10.1016/j.jbc.2021.100722> PMID: 33932402
- [34] Inukai T, Kaji S, Kataoka H. Analysis of nicotine and cotinine in hair by on-line in-tube solid-phase microextraction coupled with liquid chromatography-tandem mass spectrometry as biomarkers

- of exposure to tobacco smoke. *J Pharm Biomed Anal* 2018; 156: 272-7.  
<http://dx.doi.org/10.1016/j.jpba.2018.04.032> PMID: 29729641
- [35] Trüeb RM. The impact of oxidative stress on hair. *Int J Cosmet Sci* 2015; 37(S2) (Suppl. 2): 25-30.  
<http://dx.doi.org/10.1111/ics.12286> PMID: 26574302
- [36] Kim T, Kang J. Association between dual use of e-cigarette and cigarette and chronic obstructive pulmonary disease: An analysis of a nationwide representative sample from 2013 to 2018. *BMC Pulm Med* 2021; 21(1): 231.  
<http://dx.doi.org/10.1186/s12890-021-01590-8> PMID: 34256746
- [37] Tjahyadi D, Parwanto E. Effects of low-dose filtered kretek cigarette smoke on bronchial smooth muscle in male Sprague-Dawley rats. *Univ Med* 2023; 42: 259-71.  
<http://dx.doi.org/10.18051/UnivMed.2023.v42:259-271>
- [38] Tjahyadi D, Parwanto E, Amalia H, Digambiro RA, Edy HJ, Oladimeji AV. Decreased density of pyramidal cells in the cerebral cortex, and Purkinje cells in the cerebellar cortex of Sprague-Dawley rats after being exposed to filtered kretek cigarette smoke. *J Bio Res* 2023; 96(1): 1-6.  
<http://dx.doi.org/10.4081/jbr.2023.10757>
- [39] Burgel P-R, Martin F, Frijal A. Dysfunctional lung anatomy and small airways degeneration in COPD. *Int J Chron Obstruct Pulmon Dis* 2013; 8: 7-13.  
<http://dx.doi.org/10.2147/COPD.S28290> PMID: 23319856
- [40] Chookliang A, Sricharoenvej S, Lanlua P. Alterations in the bronchioles of rat lungs with chronic diabetes. 2019. Available From:  
[http://conf.ejikei.org/ICTSS/2019/proceedings/materials/proc\\_files/GS/ICTSS2019\\_GS\\_A007/CameraReady\\_ICTSS2019\\_GS\\_A007\\_Ms.Chookliang.pdf](http://conf.ejikei.org/ICTSS/2019/proceedings/materials/proc_files/GS/ICTSS2019_GS_A007/CameraReady_ICTSS2019_GS_A007_Ms.Chookliang.pdf)
- [41] Yanko R, Levashov M, Chaka E, Safonov S. Morphofunctional state of the lungs respiratory part in normotensive and hypertensive rats after combined exposure to intermittent hypoxia and melatonin. *J Educ Health Sport* 2021; 11(1): 56-68.  
<http://dx.doi.org/10.12775/JEHS.2021.11.01.006>
- [42] Whitsett JA. Airway epithelial differentiation and mucociliary clearance. *Ann Am Thorac Soc* 2018; 15 (Suppl. 3): S143-8.  
<http://dx.doi.org/10.1513/AnnalsATS.201802-128AW> PMID: 30431340
- [43] Zhang H, Yu W, Ji L, *et al.* Guifu dihuang pills ameliorated mucus hypersecretion by suppressing Muc5ac expression and inactivating the ERK-SP1 pathway in lipopolysaccharide/cigarette smoke-induced mice. *Evid Based Complement Alternat Med* 2021; 2021: 1-15.  
<http://dx.doi.org/10.1155/2021/9539218> PMID: 34777538
- [44] Duan J, Cheng W, Zeng Y, *et al.* Characteristics of Patients with chronic obstructive pulmonary disease exposed to different environmental risk factors: A large cross-sectional study. *Int J Chron Obstruct Pulmon Dis* 2020; 15: 2857-67.  
<http://dx.doi.org/10.2147/COPD.S267114> PMID: 33192059
- [45] Safitri W, Martini S, Artanti KD, Li CY. Smoking from a younger age is the dominant factor in the incidence of chronic obstructive pulmonary disease: Case-control study. *Int J Environ Res Public Health* 2021; 18(11): 6047.  
<http://dx.doi.org/10.3390/ijerph18116047> PMID: 34199771
- [46] Seton-Rogers S. Complex effects of tobacco on lung tissue. *Nat Rev Cancer* 2020; 20(4): 199.  
<http://dx.doi.org/10.1038/s41568-020-0249-y> PMID: 32112043
- [47] Seiler CL, Song JM, Kotandeniya D, *et al.* Inhalation exposure to cigarette smoke and inflammatory agents induces epigenetic changes in the lung. *Sci Rep* 2020; 10(1): 11290.  
<http://dx.doi.org/10.1038/s41598-020-67502-8> PMID: 32647312
- [48] Aredo JV, Luo SJ, Gardner RM, *et al.* Tobacco smoking and risk of second primary lung cancer. *J Thorac Oncol* 2021; 16(6): 968-79.  
<http://dx.doi.org/10.1016/j.jtho.2021.02.024> PMID: 33722709
- [49] Vinay S, Dharmashekara C, Prasad A. Smoking carcinogens and lung cancer-a review. *Asian J Pharm Clin Res* 2021; 14(1): 5-12.  
<http://dx.doi.org/10.22159/ajpcr.2021v14i1.39811>
- [50] Nuryunarsih D, Lewis S, Langley T. Health risks of Kretek Cigarettes: A systematic review. *Nicotine Tob Res* 2021; 23(8): 1274-82.  
<http://dx.doi.org/10.1093/ntr/ntab016> PMID: 33503247
- [51] Sadia H, Ahmad Bhinder M, Irshad A, *et al.* Determination of expression profile of p53 gene in different grades of breast cancer tissues by real time PCR. *Afr Health Sci* 2020; 20(3): 1273-82.  
<http://dx.doi.org/10.4314/ahs.v20i3.32> PMID: 33402975
- [52] Shi D, Jiang P. A different facet of p53 Function: Regulation of immunity and inflammation during tumor development. *Front Cell Dev Biol* 2021; 9(762651): 762651.  
<http://dx.doi.org/10.3389/fcell.2021.762651> PMID: 34733856
- [53] Singh R, Patel K, Patel J, Patel P. Association of interactions between Metabolic 'Caretaker' Genes, p53, MDM2, and Tobacco Use with the Risk of Oral Cancer: A multifactor dimensionality reduction approach. *Asian Pac J Cancer Prev* 2023; 24(4): 1231-7.  
<http://dx.doi.org/10.31557/APJCP.2023.24.4.1231> PMID: 37116145
- [54] Parwanto MLE, Wratsangka R, Guyansyah A, Anggraeni K. Mutation of the Fas-promoter-670 gene, AA to GA in the normal cervix-epithelial-cells of high risk Indonesian mother: A case report. *Bali Med J* 2019; 8(1): 360-4.  
<http://dx.doi.org/10.15562/bmj.v8i1.1313>
- [55] Parwanto E, Wratsangka R, Guyansyah A, *et al.* The change of cell biometric and its nucleus on cervical-squamous-epithelial-cell with GA genotype of Fas-promoter-670 gene, high-risk human papillomavirus and *Candida* species infection: A case report. *Bali Med J* 2021; 10(1): 74-81.  
<http://dx.doi.org/10.15562/bmj.v10i1.2138>

**DISCLAIMER:** The above article has been published, as is, ahead-of-print, to provide early visibility but is not the final version. Major publication processes like copyediting, proofing, typesetting and further review are still to be done and may lead to changes in the final published version, if it is eventually published. All legal disclaimers that apply to the final published article also apply to this ahead-of-print version.



PERMOHONAN PENGIRIMAN UANG  
REMITTANCE APPLICATION

BERLAKU KETENTUAN DI BALIK INI  
SUBJECT TO CONDITION REVERSE

Tgl. / Date 15/07/2024

Nama & Alamat Pengirim :  
Remitter Name & Address  
Edy Parwanto  
Jl. Garuda V Blok D6 No 10  
PAPAN MAS MANGUN JAYA  
TAMBUN SELATAN, DEKSI  
Ac No. JAWA BARAT, INDONESIA

Kepada / To  
PT. Bank Negara Indonesia (Persero) Tbk  
Cabang / Branch

1.  Harap kirirkan uang dengan  
Please remit by  Telex / Swift  Pos / Mail \*)
2.  Harap terbitkan draft  
Please issue draft

3. Penerima / Beneficiary  
● Nama/Name \*\* : Bentham Science Publishers Ltd (PTE)  
● Alamat/Address : Executive Suite Y2, PO Box 7917  
SAIF Zone, Sharjah  
United Arab Emirates, 100385232200003  
Telp. +97165571132 Fax +97165571134  
( Kota/City ) ( Negara/Country )  
● Rekening/Account No. : 012.001.225454 IBAN : AE 53038000012001225454  
● Pada/With Bank : National Bank of Fujairah PJSC, Bank Street Dubai,  
PO Box 2979, Dubai, United Arab Emirates.  
( Kota/City ) ( Negara/Country )

4. Ongkos Bank Koresponden (OKB) atas beban  
Correspondent Bank Charger (CBC) for account \*)  Penerima Beneficiary  Pengirim Remitter

5. Keterangan pembayaran : Publication Fee BMS-TORMJ-2023-19 | BSP-19173  
Payment detail : How doses of kretek cigarette smoke alter rat lung histomeric, and overexpression of the p53 gene "

6. Jumlah VA / CCY : 1005 USD  
Amount : One thousand and five DOLLAR  
dengan huruf/in words

7. Sumber dana \*\*\*  
8. Penggunaan dana \*\*\*

DIISI OLEH BANK / FILLED BY BANK

	Valas	Rupiah
Nominal	\$ 1005 USD	Rp 15.819.050,-
Komisi / Comm		Rp 35.000,-
Ongkos / Charges		Rp 395.250,-
OBK / CBC *)	3,75 USD	
Kurs / Rate	1 USD = Rp 15.810,-	
Total		Rp 16.319.300,-
Dng. Huruf / In Word		Enam Belas Juta Tiga Ratus Sembilan Belas Ribu Tiga Ratus Rupiah

- Setoran / Cover \*) :  Tunai Cash  Debit rekening kami Debit my/our account  Lain-lain Others

Disetujui / Accepted  
PT Bank Negara Indonesia (Persero) Tbk  
Ref. No. :  
( )  
Pengirim / Remitter  
( Edy Parwanto )

\*) Pilihan baik tanda / Choice marked   
\*\*) Bagi Non nasabah untuk transaksi di atas Rp. 100.000.000,- agar menyerahkan fotocopy identitas  
\*\*\*) Untuk transaksi di atas Rp. 100.000.000,-

PT. BANK NEGARA INDONESIA (Persero), Tbk  
CABANG : UGM YOGYA

IBOC - Maintenance (S10

Teller ID : 83289  
Date : 15/03/2024  
Time : 11:01:24

Sender's Reference:  
:20:S10UGM00035624  
Bank Operation Code:  
:23B:CRED  
Value Date/Currency/Interbank Settled Amount:  
:32A:240315USD1005,  
Ordering Customer:  
:50K:/0000000117163667

ML EDY PARWANTO  
JL GARUDA V BLOK D6 NO 10 PAPAN MAS  
MANGUNJAYA, TAMBUN SELATAN, BEKASI  
JAWA BARAT, INDONESIA

Ordering Institution:  
:52A:BNINIDJAXXX  
Account With Institution:  
:57D:NATIONAL BANK OF FUJAIRAH PJSC  
BANK STREET DUBAI, P.O. BOX 2979  
DUBAI, UNITED ARAB EMIRATES.

NBFUAEAFDXB  
Beneficiary Customer:  
:59:/AE520380000012001225454  
BENTHAM SCIENCE PUBLISHER LTD (FZE)  
EXECUTIVE SUITE Y2, P.O. BOX  
7917 SAIF ZONE, SHARJAH, UNITED  
ARAB EMIRATES 100385232200003

Remittance Information:  
:70:/PURP/P1107/EDUCATIONAL SERVICES/  
PUBLICATION FEE BMS-TORMJ-2023-19  
BSP-19173 LOW DOSES OF KRETEK  
CIGARETTE SMOKE ALTERED RAT LUNG

Details Of Charges:  
:71



NO. TRX. : 83289 106531 021031 BFMI 15/03/2024 11:04:09  
NO. REK. : 117163667 ML EDY PARWANTO  
JUMLAH : IDR 395.250,00- TRANSFER KE  
228 - IIGM YOGYA

1

NO. TRX. : 83289 106531 021031 BFMI 15/03/2024 11:04:09  
NO. REK. : 228840482010001 Pendapatan Restitusi Biaya ORK  
JUMLAH : USD 25,00 NPB MTSC. DE  
228 - IIGM YOGYA

1





REFERENCE : S10UGM00035624 /

NO. TRX. : 83289 974554 96963 TRAN 15/03/2024 10:06:50  
NO. REK. : 000000117163667 MI. EDY PARWANTO  
Jumlah : IDR 35,000- 1568  
228 - UGM YOGYA

A

NO. TRX. : 83289 974554 96963 TRAN 15/03/2024 10:06:50  
NO. REK. : 228360420801001 PENDAPATAN PROPTST KII  
Jumlah : IDR 35,000 1568  
228 - UGM YOGYA

A

NO. TRX. : 83289 974554 96963 TRAN 15/03/2024 10:06:50  
NO. REK. : 000000117163667 MI. EDY PARWANTO  
Jumlah : IDR 15,889,050- 1568  
228 - UGM YOGYA

A

NO. TRX. : 83289 974554 96963 TRAN 15/03/2024 10:06:50  
NO. REK. : 228840200101001 KII YAKTR  
Jumlah : USD 1,005 1568  
228 - UGM YOGYA

A





# Source details

## Open Respiratory Medicine Journal

Scopus coverage years: from 2009 to Present

Publisher: Bentham Science Publishers

ISSN: 1874-3064

Subject area: Medicine: Pulmonary and Respiratory Medicine

Source type: Journal

CiteScore 2022

1.6



SJR 2022

0.549



SNIP 2022

0.568



[View all documents >](#)

[Set document alert](#)

[Save to source list](#)

[CiteScore](#) [CiteScore rank & trend](#) [Scopus content coverage](#)

### Improved CiteScore methodology



CiteScore 2022 counts the citations received in 2019-2022 to articles, reviews, conference papers, book chapters and data papers published in 2019-2022, and divides this by the number of publications published in 2019-2022. [Learn more >](#)

CiteScore 2022

$$1.6 = \frac{80 \text{ Citations } 2019 - 2022}{51 \text{ Documents } 2019 - 2022}$$

Calculated on 05 May, 2023

CiteScoreTracker 2023

$$1.7 = \frac{91 \text{ Citations to date}}{54 \text{ Documents to date}}$$

Last updated on 05 April, 2024 • Updated monthly

## CiteScore rank 2022

Category	Rank	Percentile
Medicine		
└ Pulmonary and Respiratory Medicine	#106/144	26th

[View CiteScore methodology >](#) [CiteScore FAQ >](#) [Add CiteScore to your site](#)

---

## About Scopus

[What is Scopus](#)

[Content coverage](#)

[Scopus blog](#)

[Scopus API](#)

[Privacy matters](#)

## Language

[日本語版を表示する](#)

[查看简体中文版本](#)

[查看繁體中文版本](#)

[Просмотр версии на русском языке](#)

## Customer Service

[Help](#)

[Tutorials](#)

[Contact us](#)

---

## ELSEVIER

[Terms and conditions ↗](#) [Privacy policy ↗](#)

All content on this site: Copyright © 2024 Elsevier B.V. ↗, its licensors, and contributors. All rights are reserved, including those for text and data mining, AI training, and similar technologies. For all open access content, the Creative Commons licensing terms apply. We use cookies to help provide and enhance our service and tailor content. By continuing, you agree to the use of cookies ↗.

