# Resume mail Korespondensi (Rev)

## Re: Revision Required [BaliMedJ] [Manuscript ID:4412]

Editor Bali Medical Journal <editorbalimedicaljournal@gmail.com>
Mon 5/8/2023 10:27 AM
To: Selamat Budijitno <selamatbdr_undiplecturer@hotmail.com>

1 attachments (224 KB)
Letter of Acceptance 4412 Budijitno S.pdf;

Dear Author

Attached below is Letter of Acceptance for your article entitle: **"Impact of abelmoschus esculentus fruit extract on endonuclease_g and apoptosis index in adenocarcinoma mammae. A laboratory experiment: rats given chemotherapy AC"**

Please kindly check it first, and inform us if there is any mistake in this LoA.

Warm regards,

Editor BaliMedJ

On Mon, May 8, 2023 at 11:23AM Editor Bali Medical Journal <editorbalimedicaljournal@gmail.com> wrote:

> Dear Authors
>
> We would like to inform you that the revised version of the manuscript ID: 4412 has been well received.
>
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> With this email attached several documents of your submitted article**:**
>
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>
> **Please let us know if you are already satisfied** with the current final revised manuscript. If you approved this manuscript, your article will be processed for the galley version and published in Bali Medical Journal.
>
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>
> We're looking forward to your progress, congratulations and good luck.
>
> Best Regards
> Editorial Team

On Sat, Apr 29, 2023 at 12:49PM Selamat Budijitno <selamatbdr_undiplecturer@hotmail.com> wrote:

Dear Editor,
Thank you. I look forward to hearing from you.

Best regard

Selamat Budijitno

**From:** Editor Bali Medical Journal <editorbalimedicaljournal@gmail.com>
**Sent:** Friday, April 28, 2023 8:22 PM
**To:** Selamat Budijitno <selamatbdr_undiplecturer@hotmail.com>
**Subject:** Re: Revision Required [BaliMedJ] [Manuscript ID:4412]

Dear Authors,

We have received your payment and we would like to inform you that your manuscript is now currently being processed by our reviewer and editor.
Please patiently wait until we send you the revised version of your manuscript.

Thank you for trusting us with your hard work.

Best regards
Editorial Team

On Fri, Apr 28, 2023 at 11:22AM Selamat Budijitno <selamatbdr_undiplecturer@hotmail.com> wrote:

Dear Editor,
In the following I will send the result of editing my manuscript, and I attach the result of the SPSS output and cover letter, please correct and edit if necessary.
Thank you for the suggestion for improvement to my manuscript.

**From:** Selamat Budijitno <selamatbdr_undiplecturer@hotmail.com>
**Sent:** Wednesday, April 26, 2023 8:12 PM
**To:** Editor Bali Medical Journal <editorbalimedicaljournal@gmail.com>
**Subject:** Re: Revision Required [BaliMedJ] [Manuscript ID:4412]

dear editor,

Thankyou for your review. I will revise the manuscript according to required structured by using Publon review checklist. As soon as I already done the revision, I will re-submit the revised manuscript

best regards,

dr. Selamat

**From:** Editor Bali Medical Journal <editorbalimedicaljournal@gmail.com>
**Sent:** Sunday, April 23, 2023 9:00 AM
**To:** selamatbdr_undiplecturer@hotmail.com <selamatbdr_undiplecturer@hotmail.com>
**Subject:** Revision Required [BaliMedJ] [Manuscript ID:4412]

Dear Authors,
Thank you for submitting your article entitled: "**Impact of abelmoschus esculentus fruit extract on endonuclease_g and apoptosis index in adenocarcinoma mammae. A laboratory experiment: rats given chemotherapy AC"**

Based on our author guidelines, Your article fulfilled the minimal required structure,
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In order to have a better-structured article, we suggest you edit based on a checklist,
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According to the new International regulation, please fulfill the requirements below:

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Ponten et al., showed that fasciocutaneus flap could be utilized to cover lower leg soft tissue defects.[1]

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1. Pontén B. The fasciocutaneous flap: its use in soft tissue defects of the lower leg. *Br J Plast Surg*. 1981;34(2):215–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7236984

Please revise your article with the missing details, and send it back to us in **3 days (April 26th, 2023).**

In addition, I do need to remind you that Bali Medical Journal is free to submit and Open Access for our readers. However, if your manuscript is accepted for publication, as the author, you will be charged **1,600 USD USD** for APC included for proofreading, editing Formatting, Lay outing, and Galey.

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Warm regards,

Editorial Board Member

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Lampiran :

Artikel yang disubmit :

**Impact of abelmoschus esculentus fruit extract on endonuclease_g and apoptosis index in adenocarcinoma mammae. A laboratory experiment: rats given chemotherapy AC**

Budijitno S[1], Prawiro SP[1], Nugroho EA[1], Alwi L[2],PAT Adiputra
[1] Surgical Department, Faculty of Medicine Diponegoro University
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Cancer in women with the highest incidence is breast cancer. Adriamycin-cyclophosphamide (AC) first-line chemotherapy has good results, but its efficacy is not optimal. The fruit lectin substance Abelmoschus esculentus has an anti-cancer effect so it is widely used as a chemotherapy supplement.The aim of study to prove Abelmoschus esculentus extract can increase the apoptotic index against chemotherapy Method. In vivo laboratory trial study, using Sprague Dawley female rats aged 28 days with mammary adenocarcinoma induced by DMBA. There are 4 groups, namely; control (K): placebo, treatment1 (P1): AC chemotherapy (adriamycin1.5 mg and cyclophosphamide 15 mg), treatment2 (P2): Abelmoschus esculentus extract 150 mg/kgBB/day, and treatment3 (P3): a combination of AC and Abelmoschus esculentus extract. Results**.** The highest levels of Endonuclease_G (EndoG) and apoptosis were obtained in the P3 group of (38.66 ± 0.73) and (21.03 ± 0.69), respectively. Combination Abelmoschus esculentus extract to chemotherapeutic agents can increase the anticancer effect by significantly increasing EndoG expression and apoptosis index ($p<0.05$) compared to other groups.
The Conclusion Extract from Abelmoschus esculentus fruit was able to increase the apoptotic response to in vivo in-cyclophosphamide adriamis chemotherapy as indicated by the high expression of EndoG and the apoptotic index.
Keywords: A esculentus , Adenocarcinoma mammae, Endo_G, Apoptosis

**Introduction**

Cancer in women with the highest incidence is breast cancer. The main therapeutic measures consist of operative management, radiation, and chemotherapy. Chemotherapy is a therapeutic option in advanced breast cancer, some of the most commonly used combination regimens, including: fluorouracil, adriamycin, and cyclophosphamide (FAC); fluorouracil, epirubicin, and cyclophosphamide (FEC); adriamycin and cyclophosphamide (AC); and cyclophosphamide, methotrexate, and fluorouracil (CMF). before surgery, neoadjuvant chemotherapy can improve outcomes in locally advanced breast cancer. [1-5] The efficacy of cancer chemotherapy always wants to be improved, one way is to increase apoptosis. [6] Many natural ingredients have been studied which have the effect of increasing apoptosis of breast cancer cells. [7] In tropical and subtropical regions, the fruit of Abelmoschus esculentus is often cultivated. Seed extracts of Abelmoschus esculentus and fruit of Abelmoschus esculentus can function as anti-free radicals and anti-cancer. [8] Toxicity Extracts from seeds and fruits of Abelmoschus esculentus are quite safe to use. High levels of isoquercentin and quercentin-3-O-gentiobiose, flavonoids, lectins, are often explored for their anti-cancer benefits. [9-13]

**Method**

In vivo laboratory trial with post test only control group design. A total of 24 female Sprague Dawley rats aged 28 days with a body weight of 100-150 grams were induced by DMBA of 20 mg/kg BW until carcinoma mammae appeared, randomly alocation into 4 groups, Control group K, not given therapy, P1: received AC chemotherapy (adriamycin 1.5 mg/time and cyclophosphamide 15 mg/time), P2: given Abelmoschus esculentus fruit extract 150 mg/kgBB/day, P3: given AC chemotherapy plus Abelmoschus esculentus fruit extract as much as 150 mg/kgBB. Approval from the medical and health research ethics committee, given from the Faculty of Medicine, Diponegoro University, Semarang, Indonesia. The dried Abelmoschus esculentus simplicia was then powdered until smooth and sieved. Abelmoschus esculentus fruit powder is soaked for 24 hours, the filtrate is taken, then a thick extract is obtained using a rotary evaporator. Tumor tissue from rats was paraffinized and examined by immunohistochemistry to see the expression of apoptotic EndoG and Indes. The interpretation of the results is carried out with >95% agreement. Then the data is tabulated. Descriptive analysis and ANOVA test, post

hoc Bonferroni test were carried out. The significance limit of P <0.05 with a CI of 95%. Data analysis using SPSS version 26.0 for Windows.

**Results**

From 35 DMBA-induced mice, 4 mice did not develop tumors, 7 mice only had adenomas. Twenty four mice were randomized into 4 groups. Groups P1, P2 and P3 showed greater expression of EndoG than group K. Groups P1 and P3 had higher expression of EndoG than group P2. The most optimal combination in increasing EndoG occurred in group (3). Groups P1, P2 and P3 showed a higher apoptotic index than group K. Groups P1 and P3 showed a higher apoptotic index than group P2. The most optimal combination in increasing apoptotic index occurred in group (3). There is a fairly strong correlation between increased EndoG expression and the apoptosis index *(p=0.001, r=0.985)*

**Discussion**

Mitochondrial Endonuclease G, is an enzyme that in humans is encoded by the EndoG gene.[14,15] This protein primarily participates in caspase-independent apoptosis via DNA degradation when translocating from the mitochondrion to nucleus under oxidative stress.[16] As a result, EndoG has been implicated in cancer cell. The protein encoded by this gene is a nuclear encoded endonuclease that is localized in the mitochondrial intermembrane space. [14,17] EndoG is released from the mitochondrion and migrates to the nucleus, where it degrades chromatin with the help of other nuclear proteins. [16,18 ,19] Under normal conditions, EndoG remains bound to Hsp70 and CHIP; however, when undergoing oxidative stress, EndoG dissociates from Hsp70 and CHIP and translocates to the nucleus, where it degrades DNA to effect apoptosis. In addition to DNA degradation, EndoG also stimulates inhibitors of apoptosis proteins (IAPs) to target proteins for proteasomal degradation.[11,20] The lectins contained in Abelmoschus esculentus have been extensively studied for their anti-cancer effects. Lectins can induce apoptosis by lectins starting with their interaction with sugar-binding receptors on the plasma membrane and endocytosis occurs. Lectin vesicles go to mitochondria to generate reactive oxygen species (ROS) rip off mitochondrial membrane and release EndoG into the cytoplasm. And then EndoG dissociates from Hsp70 and CHIP and translocates to the nucleus, where it degrades DNA to effect apoptosis. 15 days. The results found can reduce the diameter of the tumor and reduce the amount of density of breast cancer vascularization.

**Conclusion**

Abelmoschus esculentus extract can increase the apoptotic index against chemotherapy in vivo in mice given AC chemotherapy via the EndoG pathway.

**Ethical approval**
**Funding**
**Conflict of Interest**
**Author contribution**

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**Commentary**

Dear Sir/Madam,
Here are some commentaries to the manuscript entitled
**"Impact of Abelmoschus esculentus fruit extract on endonuclease_G and apoptosis index in adenocarcinoma mammae. A laboratory experiment: rats given chemotherapy AC"**

| No. | Section | Commentary |
|---|---|---|
| A | Title and Affiliation | 1. The affiliation of each author needs to be appropriately written by suggesting their University (in Indonesia), Institution, or any other relevant background |
| B | Abstract | 1. Please follow the BMRAD methods in written the abstract of research articles<br>2. The keywords supposedly better written less than 5 words and sorted alphabetically |
| C | Introduction | Consider determining the primary outcome expected to be found in this study |
| D | Methods | Method was clear without revision |
| E | Results | The tables are not written adequately based on author guidelines. Consider fixing this issue |
| F | Discussion | Do not repeat the results in the discussion section. Please focuses on the discrepancy between the recent findings and the previous studies |
| G | Conclusion | Please provide information regarding conflict of interest, funding, author contribution, and ethics statement following conclusion |
| H | Table, figure and Reference | 1. Table and figure descriptions not appropriate with the guidelines |
| I | Others | 1. There were numerous grammatical errors |

INDONESIAN PHYSICIAN FORUM & INDONESIA COLLEGE OF SURGEONS, INDONESIA
Bali Medical Journal
ISSN: 2089-1180, 2302-2914

COVER LETTER

Date: April 28th, 2023

Journal name: BALI MEDICAL JOURNAL, Article type: A Laboratory Experiment

I am enclosing herewith a manuscript entitled " **Impact of abelmoschus esculentus fruit extract on endonuclease_g and apoptosis index in adenocarcinoma mammae. A laboratory experiment: rats given chemotherapy AC**" for publication in the Bali Medical Journal for possible evaluation. The Corresponding author of this manuscript is Selamat Budijitno and the contribution of the authors as mentioned below with their responsibility in the research.

Author full Name and email


| | | |
|---|---|---|
| 1 | Selamat Budijitno | selamatbdr_undiplecturer@hotmail.com |
| 2 | Satrio Putra Prawiro | satrioputra20@gmail.com |
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| | Contributor 1 | Contributor 2 | Contributor 3 | Contributor 4 | Contributor 5 |
|---|---|---|---|---|---|
| Concepts | √ | | | | |
| Design | √ | √ | | | |
| Definition of intellectual content | √ | √ | √ | √ | √ |
| Literature search | √ | √ | √ | √ | √ |
| Clinical studies | | | | | |
| Experimental studies | √ | √ | √ | √ | |
| Data acquisition | √ | √ | | | |
| Data analysis | √ | √ | √ | √ | √ |
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Author Contribution Details (to be ticked marked as applicable)

1. UNDERTAKING

With the submission of this manuscript, I would like to undertake that:

- All authors of this Article (paper) have directly participated in the planning, execution, or analysis of this study;
  - All authors of this paper have read and approved the final version submitted;
  - The contents of this manuscript have not been copyrighted or published previously;
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- The contents of this manuscript will not be copyrighted, submitted, or published elsewhere, while acceptance by the Journal is under consideration;
- There are no directly related manuscripts or abstracts, published or unpublished, by any authors of this paper;
- My Institute's Diponegoro University and Semarang State University representatives are fully aware of this submission.

## 2. RESEARCH AND MANUSCRIPT-RELATED DETAILS

The submitted manuscript is a laboratory experiment

The research project was conducted under the supervision of:
Research and Ethics Committee of the Faculty of Medicine Diponegoro University, Indonesia protocol number: 140/EC/H/FK-UNDIP/XII/2022.

## 3. GRANTS OR FUNDING INFORMATION

Nobody provided funding for this research project. The authors are responsible for all costs associated with this study.

## 4. CONFLICT OF INTEREST

The authors declare no conflicts of interest.

Date: April 28th, 2023
Corresponding author

Selamat Budijitno

Artikel dengan revisi :

# Impact of abelmoschus esculentus fruit extract on endonuclease_g and apoptosis index in adenocarcinoma mammae. A laboratory experiment: rats given chemotherapy AC

Budijitno S[1], Prawiro SP[1], Nugroho EA[1], Alwi L[2], Putu Anda Tusta Adiputra[3]
[1] Surgical Department, Faculty of Medicine Diponegoro University, Semarang, Indonesia
[2] Surgical Department, Faculty of Medicine Semarang State University, Semarang, Indonesia
[3] Surgical Department, Faculty of Medicine Udayana University, Denpasar, Indonesia

Email: selamatbdr_undiplecturer@hotmail.com

**Background.** Cancer in women with the highest incidence is breast cancer. Adriamycin-cyclophosphamide (AC) first-line chemotherapy has good results, but its efficacy is not optimal. The fruit lectin substance Abelmoschus esculentus has an anti-cancer effect so it is widely used as a chemotherapy supplement.The aim of study to prove Abelmoschus esculentus extract can increase the apoptotic index against chemotherapy
**Method.** In vivo laboratory trial study, using Sprague Dawley female rats aged 28 days with mammary adenocarcinoma induced by DMBA. There are 4 groups, namely; control (K): placebo, treatment1 (P1): AC chemotherapy (adriamycin1.5 mg and cyclophosphamide 15 mg), treatment2 (P2): Abelmoschus esculentus extract 150 mg/kgBB/day, and treatment3 (P3): a combination of AC and Abelmoschus esculentus extract.
**Results.** The highest levels of Endonuclease_G (EndoG) and apoptosis were obtained in the P3 group of (38.66 ± 0.73) and (21.03 ± 0.69), respectively. Combination Abelmoschus esculentus extract to chemotherapeutic agents can increase the anticancer effect by significantly increasing EndoG expression and apoptosis index ($p<0.05$) compared to other groups.
**Conclusion.** Extract from Abelmoschus esculentus fruit was able to increase the apoptotic response to in vivo in-cyclophosphamide adriamis chemotherapy as indicated by the high expression of EndoG and the apoptotic index.
Keywords: Abelmoschus esculentus , Adenocarcinoma mammae, Endo_G, Apoptosis

## Introduction

Cancer in women with the highest incidence is breast cancer. The main therapeutic measures consist of operative management, radiation, and chemotherapy. Chemotherapy is a therapeutic option in advanced breast cancer, some of the most commonly used combination regimens, including: fluorouracil, adriamycin, and cyclophosphamide (FAC); fluorouracil, epirubicin, and cyclophosphamide (FEC); adriamycin and cyclophosphamide (AC); and cyclophosphamide, methotrexate, and fluorouracil (CMF). before surgery, neoadjuvant chemotherapy can improve outcomes in locally advanced breast cancer. [1-5] The efficacy of cancer chemotherapy always wants to be improved, one way is to increase apoptosis. [6] Many natural ingredients have been studied which have the effect of increasing apoptosis of breast cancer cells. [7] In tropical and subtropical regions, the fruit of Abelmoschus esculentus is often cultivated. Seed extracts of Abelmoschus esculentus and fruit of Abelmoschus esculentus can function as anti-free radicals and anti-cancer. [8] Toxicity Extracts from seeds and fruits of Abelmoschus esculentus are quite safe to use. High levels of isoquercentin and quercentin-3-O-gentiobiose, flavonoids, lectins, are often explored for their anti-cancer benefits. [9-13] The aim of study to prove Abelmoschus esculentus extract can increase the apoptotic index against chemotherapy through EndoG pathway.

## Method

In vivo laboratory trial with post test only control group design. A total of 24 female Sprague Dawley rats aged 28 days with a body weight of 100-150 grams were induced by DMBA of 20 mg/kg BW until carcinoma mammae appeared, randomly alocation into 4 groups, Control group K, not given therapy, P1: received AC chemotherapy (adriamycin 1.5 mg/time and cyclophosphamide 15 mg/time), P2: given Abelmoschus esculentus fruit extract 150 mg/kgBB/day, P3: given AC chemotherapy plus Abelmoschus esculentus fruit extract as much as 150 mg/kgBB. Approval from the medical and health research ethics committee, given from the Faculty of Medicine, Diponegoro University, Semarang, Indonesia. The dried Abelmoschus esculentus simplicia was then powdered until smooth and sieved. Abelmoschus esculentus fruit powder is soaked for 24 hours, the filtrate is taken, then a thick extract is obtained using a rotary evaporator. Tumor tissue from rats was paraffinized and examined by immunohistochemistry to see the expression of apoptotic EndoG and Indes. The interpretation of the results is carried out with >95% agreement. Then the data is tabulated. Descriptive

analysis and ANOVA test, post hoc Bonferroni test were carried out. The significance limit of P <0.05 with a CI of 95%. Data analysis using SPSS version 26.0 for Windows.

## Results

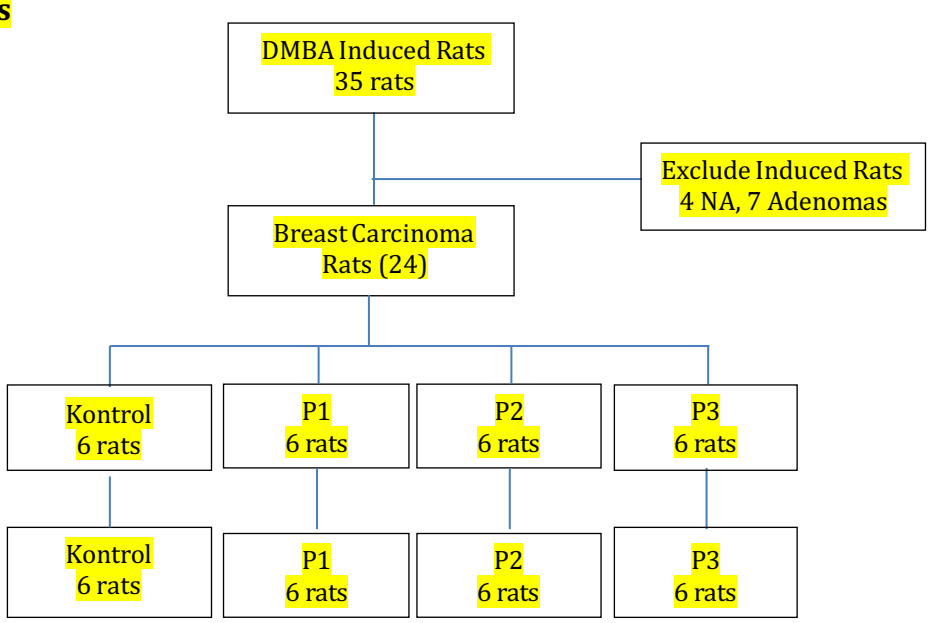


**Figure-1.** CONSORT diagram. Rats were randomly assigned to receive management either with Abelmoschus esculentus solution (treatment group) or with 0,9% NaCl (non-treatment group)

From 35 DMBA-induced rats, 4 rats did not develop tumors, 7 rats only had adenomas. Twenty four rats were randomized into 4 groups for further analysis.

## Endonuclease-G expression

The data obtained is tested for normality with Shapiro-Wilk. All the data of Endonuclease-G expression in all groups were normally distributed (p >0.05), and Homogenrity of Variance tested by Levene Test is equal variance assumed (p>0,05). The comparison among group are presented in Table-1. Using One-way ANOVA test, there is significantly difference among the treatment and the control group (p <0.001). Using the Bonferroni post hoc test, the difference between groups obtain as shown in the boxplot image (figure-2).

**Table 1.** Endonuclease-G Expression among treatment and control groups

| Grup | Endo-G | P value* |
|---|---|---|
| K | 18.8617±3.74788 | *P<0.001* |
| P1 | 28.9667±2.62524 | |
| P2 | 21.2450±4.00108 | |
| P3 | 38.6617±4.00108 | |

** = One Way ANOVA*

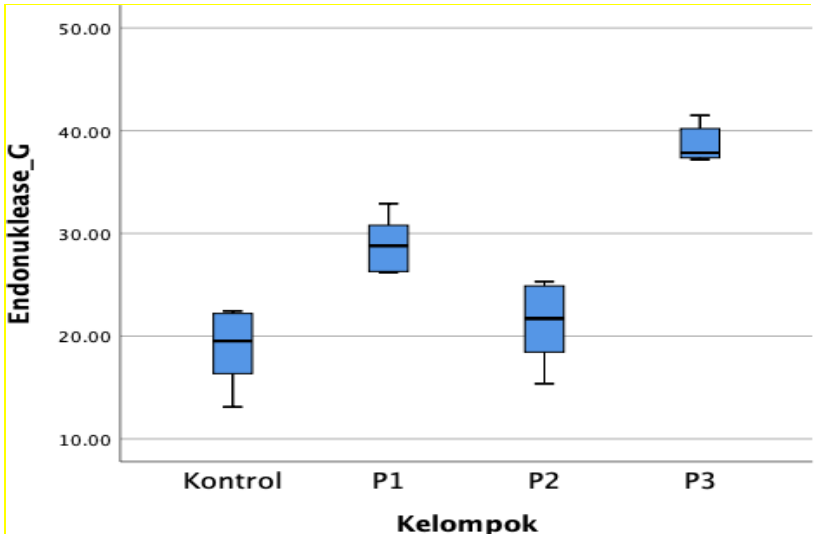


**Figure-2**, The Bonferroni post hoc test of Endonuclease-G Expression, (K vs P1, p<0.001), (K vs P2, p>0.05), (K vs P3, p<0.001), (P1 vs P2, p>0.05), (P1 vs P3, p<0.001).

Groups P1, and P3 showed greater expression of Endo-G than group K. There is no significant different between Group K and P2. Groups P1 and P3 had higher expression of Endo-G than group P2. The most optimal combination in increasing Endo-G expression occurred in group (P3).

## Apoptotic Index

The data obtained is tested for normality with Shapiro-Wilk. All the data of Apoptotic Index in all groups were normally distributed (p >0.05), and Homogenrity of Variance tested by Levene Test is equal variance assumed (p>0,05). The comparison among group are presented in Table-2. Using One-way ANOVA test, there is significantly difference among the treatment and the control group (p <0.001). Using the Bonferroni post hoc test, the difference between groups obtain as shown in the boxplot image (figure-3).

**Table-2.** Apoptotic Index among treatment and control groups

| Grup | Apoptotic Index | P value* |
|---|---|---|
| K | 2.5850±1.39920 | *P<0.001* |
| P1 | 18.1417±1.82860 | |
| P2 | 3.8600±1.30765 | |
| P3 | 21.0333±1.69827 | |

** = One Way ANOVA*

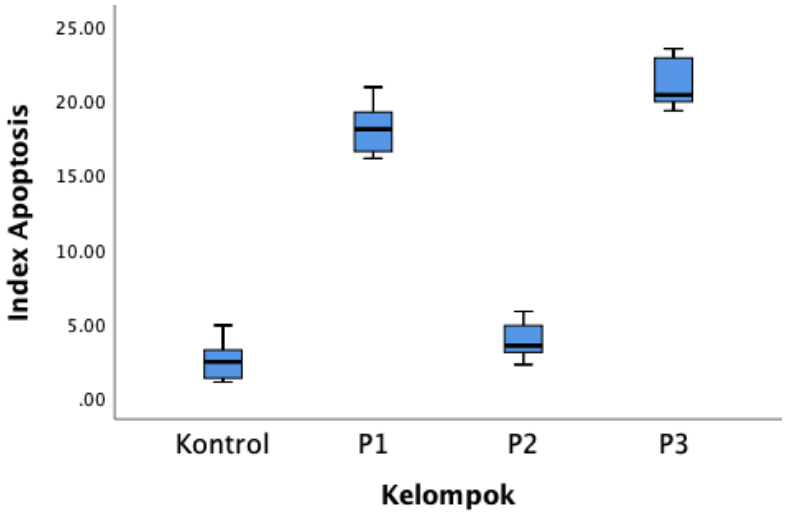


**Figure-3**, The Bonferroni post hoc test of Apoptotic Index, (K vs P1, p<0.001), (K vs P2, p>0.05), (K vs P3, p<0.001), (P1 vs P2, p>0.05), (P1 vs P3, p=0.028).

Groups P1, and P3 showed greater Apoptotic Index than group K. There is no significant different between Group K and P2. Groups P1 and P3 had higher Apoptotic Index than group P2. The most optimal combination in increasing Apoptotic Index occurred in group (P3).

**The relationship between the expression of Endo-G and Apoptotic Index in combination treatment**

The average Endo-G expression in the combination treatment group (P3) was 38.66±4.001, and the average apoptotic index in the combination treatment group (P3) was 21.03±1.698. The relationship between the Endo-G expression and apoptotic index in the combination treatment group was tested with Pearson's Correlation test. There was a fairly strong correlation between increased Endo-G expression and increased the apoptosis index *($p<0.001$, $r=0.985$)*

## Discussion

Mitochondrial Endonuclease G, is an enzyme that in humans is encoded by the EndoG gene.[14,15] This protein primarily participates in caspase-independent apoptosis via DNA degradation when translocating from the mitochondrion to nucleus under oxidative stress.[16] As a result, EndoG has been implicated in cancer cell. The protein encoded by this gene is a nuclear encoded endonuclease that is localized in the mitochondrial intermembrane space. [14,17] EndoG is released from the mitochondrion and migrates to the nucleus, where it degrades chromatin with the help of other nuclear proteins. [16,18,19] Under normal conditions, EndoG remains bound to Hsp70 and CHIP; however, when undergoing oxidative stress, EndoG dissociates from Hsp70 and CHIP and translocates to the nucleus, where it degrades DNA to effect apoptosis. In addition to DNA degradation, EndoG also stimulates inhibitors of apoptosis proteins (IAPs) to target proteins for proteasomal degradation.[11,20] The lectins contained in Abelmoschus esculentus have been extensively studied for their anti-cancer effects. Lectins can induce apoptosis by lectins starting with their interaction with sugar-binding receptors on the plasma membrane and endocytosis occurs. Lectin vesicles go to mitochondria to generate reactive oxygen species (ROS) rip off mitochondrial membrane and release EndoG into the cytoplasm. And then EndoG dissociates from Hsp70 and CHIP and translocates to the nucleus, where it degrades DNA to effect apoptosis. 15 days. The results found can reduce the diameter of the tumor and reduce the amount of density of breast cancer vascularization.

In this study it was shown that Abelmoschus esculentus extract did not have efficacy alone given without chemotherapy as the main therapy. And when given in combination with chemotherapy, there will be a very high synergy effect. This can be caused because Abelmoschus esculentus can trigger apoptosis through the Caspase and Endonuclease-G pathways, where Endonuclease-G substances and other pro-apoptotic cytokines that trigger apoptosis from the Caspase pathway are abundant in the mitochondrial double layer membrane. If chemotherapy is added, this chemotherapy agent, especially Adriamycin, will form free oxygen radicals which will accelerate the breakdown of the mitochondrial double layer membrane.[21] Thus accelerating the triggering of apoptosis via the Endonuclease-G pathway.

The limitation in this study, we did not measure the many intermediate cytokine on the apoptosis cascade, seems like BAD, BAK, IAP protein, and the role/influence of Fas Associated Death Domain

## Conclusion

Abelmoschus esculentus extract can increase the apoptotic index against chemotherapy in vivo in rats through the Endo-G pathway when given in combination with AC chemotherapy

Ethical Approval:
The animal experimental was approved by the Research and Ethics Committee of the Faculty of Medicine Diponegoro University, Indonesia (protocol number: 140/EC/H/FK-UNDIP/XII/2022).

Grants or funding information
Nobody provided funding for this research project. The authors are responsible for all costs associated with this study

Conflict of interest: the authors declare no conflicts of interest.

Author Contribution Details (to be ticked marked as applicable):

| | Contributor 1 | Contributor 2 | Contributor 3 | Contributor 4 | Contributor 5 |
|---|---|---|---|---|---|
| Concepts | √ | | | | |
| Design | √ | √ | | | |
| Definition of intellectual content | √ | √ | √ | √ | √ |
| Literature search | √ | √ | √ | √ | √ |
| Clinical studies | | | | | |
| Experimental studies | √ | √ | √ | √ | |
| Data acquisition | √ | √ | | | |
| Data analysis | √ | √ | √ | √ | √ |
| Statistical analysis | √ | √ | √ | √ | √ |
| Manuscript preparation | √ | √ | | | |
| Manuscript editing | √ | | | √ | √ |
| Manuscript review | √ | | | | √ |
| Guarantor | √ | | | | |

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**Letter of Acceptance**
01 May 2023

**Dear: Selamat Budijitno[1*], Satrio Putra Prawiro[1], Eriawan Agung Nugroho[1], Alwi L[2], Putu Anda Tusta Adiputra[3]**

[1]Surgical Department, Faculty of Medicine Universitas Diponegoro, Semarang, Indonesia
[2]Surgical Department, Faculty of Medicine Universitas Negeri Semarang, Semarang, Indonesia
[3]Surgical Department, Faculty of Medicine, Universitas Udayana Denpasar, Indonesia

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I am very excited to accept your paper entitled:
**"Impact of abelmoschus esculentus fruit extract on endonuclease_g and apoptosis index in adenocarcinoma mammae. A laboratory experiment: rats given chemotherapy AC."**
Your paper will be published in the issue of Vol. 12 Number 2, 2023.
**http://dx.doi.org/10.15562/bmj.v12i2.4412**
(Online Link: http://balimedicaljournal.org/index.php/bmj/article/view/4412).

And it usually takes 2 to 4 months for your journal to show up at Google Scholar, but if you need it fast, you may add it up manually using your google scholar account. The CrossRef and DOI number usually activate in 3 until 6 months.

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22. SJIF Journal Rank
23. Science Impact Factor (SIF)
24. Genamics
25. ASEAN Citation Index (ACI)
26. UDL-Edge (Malaysia)

**Artikel Accepted :**

**Original Article**

# Impact of Abelmoschus esculentus fruit extract on endonuclease_G and apoptosis index in adenocarcinoma mammae. A laboratory experiment: rats given chemotherapy AC

Selamat Budijitno[1*], Satrio Putra Prawiro[1], Eriawan Agung Nugroho[1], Luqman Alwi[2], Putu Anda Tusta Adiputra[3]

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**Background:** Breast cancer has the highest incidence of cancer in women. The first-line chemotherapy, Adriamycin-cyclophosphamide (AC) has good results, but its efficacy is not optimal. The fruit lectin substance Abelmoschus esculentus has an anti-cancer effect, so it is widely used as a chemotherapy supplement. The aim of the study is to prove Abelmoschus esculentus extract can increase the apoptotic index against chemotherapy

**Method:** In vivo laboratory trial study, we are using Sprague Dawley female rats aged 28 days with mammary adenocarcinoma induced by DMBA. There are 4 groups, namely; control (K): placebo, treatment1 (P1): AC chemotherapy (Adriamycin 1.5 mg and cyclophosphamide 15 mg), treatment2 (P2): Abelmoschus esculentus extract 150 mg/kgBB/day, and treatment3 (P3): a combination of AC and Abelmoschus esculentus extract.
**Results:** The P3 group had the highest levels of Endonuclease_G (EndoG) and apoptosis at (38.66 ± 0.73) and (21.03 ± 0.69), respectively. Combination of Abelmoschus esculentus extract with chemotherapeutic agents can improve the anticancer outcome by significantly increasing EndoG expression and apoptosis index ($p<0.05$) in comparison to other groups.

**Conclusion:** Extract from Abelmoschus esculentus fruit shows high apoptotic index and EndoG expression which means that this extract can increase the apoptotic response to in vivo in-cyclophosphamide adriamis chemotherapy.
Keywords: Abelmoschus esculentus, Adenocarcinoma mammae, Endo_G, Apoptosis

## INTRODUCTION

Breast cancer has the highest incidence of cancer in women. Surgical management, chemotherapy, and radiation are the main therapeutic steps. Chemotherapy is a therapeutic option in advanced breast cancer. Some of the most commonly used combination regimens, including fluorouracil, epirubicin, and cyclophosphamide (FEC); fluorouracil, adriamycin, and cyclophosphamide (FAC); cyclophosphamide, methotrexate, and fluorouracil (CMF); and adriamycin and cyclophosphamide (AC). In general, neoadjuvant chemotherapy can increase outcomes in locally advanced breast cancer before doing the surgery.[1-5]
The efficacy of cancer chemotherapy always wants to be improved, and one way is to increase apoptosis.[6] Currently, many natural materials have been studied that can increase breast cancer cell apoptosis.[7] In subtropical and tropical area, the fruit of Abelmoschus esculentus is often cultivated. Seed extracts of Abelmoschus esculentus and fruit of Abelmoschus esculentus can function as anti-free radicals and anti-cancer.[8] Toxicity Extracts from seeds and fruits of Abelmoschus esculentus are quite safe to use. High levels of isoquercentin and quercetin-3-O-gentiobiose, flavonoids, and lectins, are often explored for their anti-cancer benefits.[9-13] The aim of the study is to prove Abelmoschus esculentus extract can increase the apoptotic index against chemotherapy through the EndoG pathway.

## METHOD

This research method used a post-Test only control group design with in vivo laboratory trials. A total of 24 female Sprague Dawley rats aged 28 days with a body weight of 100-150 grams were induced by DMBA of 20 mg/kg BW until carcinoma mammae appeared, randomly allocated into 4 groups, Control group K, not given therapy, P1: received AC chemotherapy (adriamycin 1.5 mg/time and cyclophosphamide 15 mg/time), P2: given Abelmoschus esculentus fruit extract 150 mg/kgBB/day, P3: given AC chemotherapy plus Abelmoschus esculentus fruit extract as much as 150 mg/kgBB. This study was approved by the health and medical research ethics committee of the Faculty of Medicine, Diponegoro University, Semarang, Indonesia. The dried Abelmoschus esculentus simplicia was then made into

smooth powder and sifted. Abelmoschus esculentus fruit powder is soaked for 24 hours,thefiltrateistaken,andathickextract is obtained using a rotary evaporator. Tumor tissue from rats was paraffinized and examined by immunohistochemistry to see the expression of apoptotic EndoG and Indes. The interpretation of the results is carried out with >95% agreement. Then the data is tabulated. Descriptive analysis and, ANOVA test, post hoc Bonferroni test was carried out. The significance limit of P <0.05 with a CI of 95%. Data analysis using SPSS version 26.0 for Windows.

## RESULTS

From 35 DMBA-induced rats, 4 rats did not develop tumors, and 7 rats only had adenomas. Twenty-four rats were randomized into 4 groups for further analysis.

### Endonuclease-G expression

The data obtained is tested for normality with Shapiro-Wilk. All the data of Endonuclease-G expression in all groups were normally distributed (p >0.05), and the homogeneity of Variance tested by the Levene Test is equal variance assumed (p>0,05). The comparison among the group is presented in Table 1. Using the One-way ANOVA test, there is a significant difference between the treatment and the control group (p <0.001). Using the Bonferroni post hoc test, the difference between groups obtain as shown in the boxplot image (Figure 2).

Groups P1 and P3 showed greater expression of Endo-G than group K. There is no significant difference between Group K and P2. Groups P1 and P3 had higher expression of Endo-G than group P2. The most optimal combination in increasing Endo-G expression occurred in group (P3).

### Apoptotic Index

The data obtained is tested for normality with Shapiro-Wilk. All the data of the Apoptotic Index in all groups were normally distributed (p >0.05), and the homogeneity of Variance tested by the Levene Test is equal variance assumed (p>0,05). The comparison among the group is presented in Table 2. Using the One-way ANOVA test, there is a significant

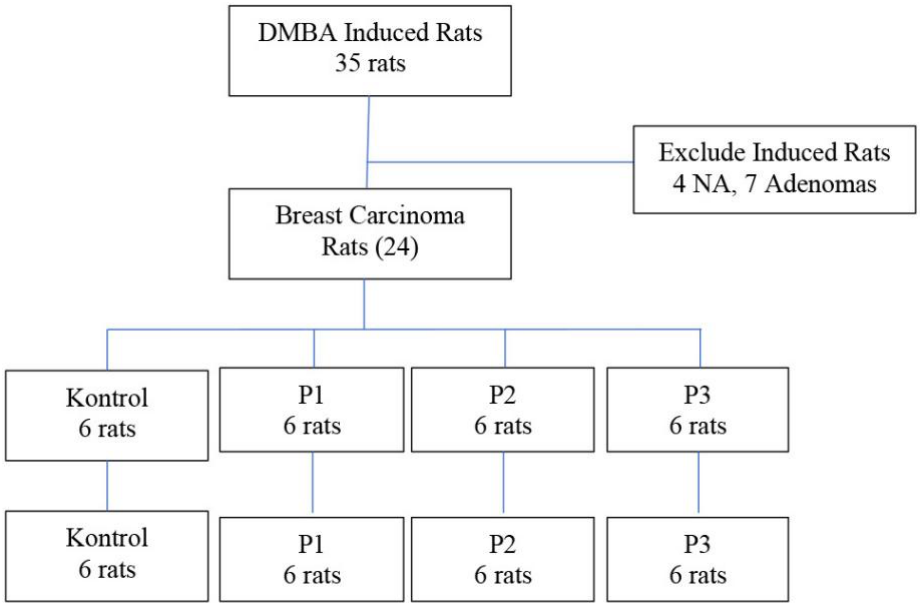


**Figure 1.** CONSORT diagram. Rats were randomly assigned to receive management either with Abelmoschus esculentus solution (treatment group) or with 0,9% NaCl (non-treatment group)

**Table 1. Endonuclease-G Expression among Treatment and control groups**

| Grup | Endo-G | P value* |
|---|---|---|
| K | 18.8617±3.74788 | |
| P1 | 28.9667±2.62524 | |
| P2 | 21.2450±4.00108 | *P<0.001* |
| P3 | 38.6617±4.00108 | |

* = *One-Way ANOVA*

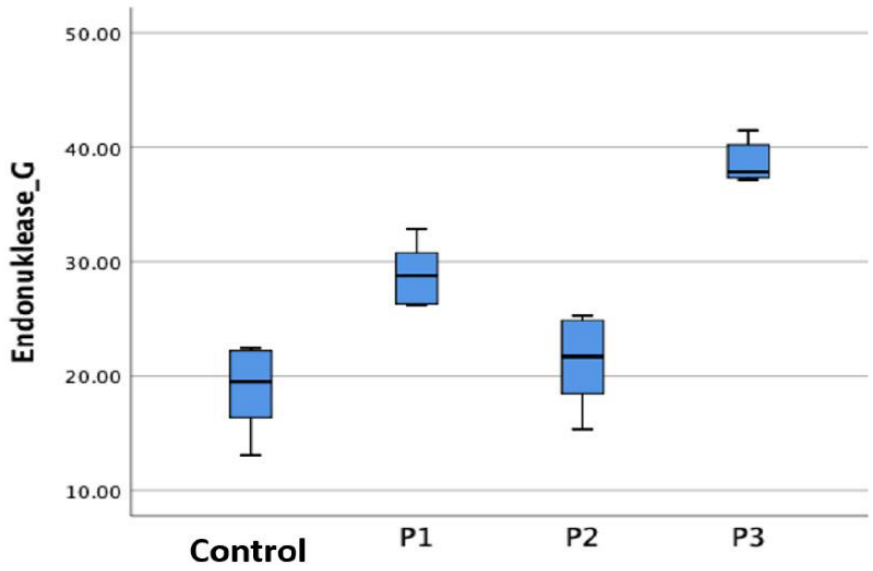


**Figure 2.** The Bonferroni post hoc test of Endonuclease-G Expression, (K vs. P1, p<0.001), (K vs. P2, p>0.05), (K vs. P3, p<0.001), (P1 vs. P2, p>0.05), (P1 vs P3, p<0.001).

**Table 2.** Apoptotic Index among Treatment and control groups

| Grup | Apoptotic Index | P value* |
|---|---|---|
| K | 2.5850±1.39920 | |
| P1 | 18.1417±1.82860 | |
| P2 | 3.8600±1.30765 | *P<0.001* |
| P3 | 21.0333±1.69827 | |

* = *One-Way ANOVA*

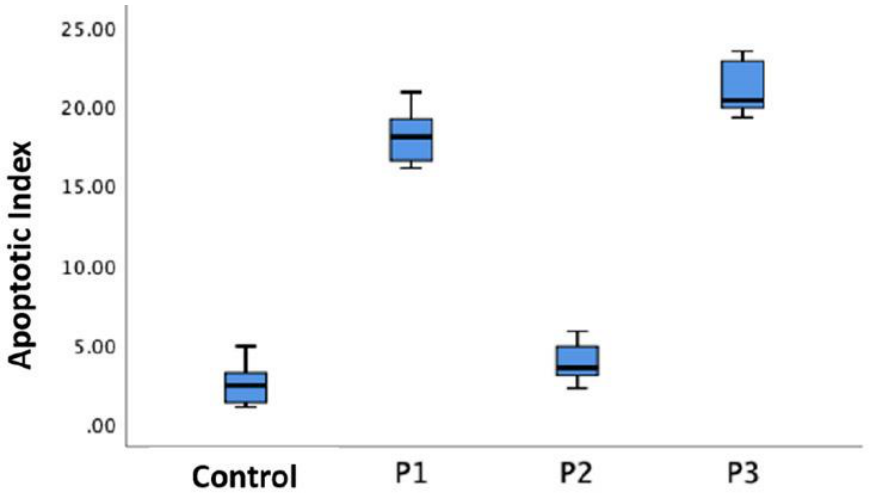


**Figure 3.** The Bonferroni post hoc test of Apoptotic Index, (K vs. P1, p<0.001), (K vs. P2, p>0.05), (K vs. P3, p<0.001), (P1 vs. P2, p>0.05), (P1 vs P3, p=0.028).

difference between the treatment and the control group (p <0.001). Using the Bonferroni post hoc test, the difference between groups obtain as shown in the boxplot image (**Figure 3**).

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### The relationship between the expression of Endo-G and Apoptotic Index in combination treatment

The average Endo-G expression in the combination treatment group (P3) was 38.66±4.001, and the average apoptotic index in the combination treatment group (P3) was 21.03±1.698. The relationship between the Endo-G expression and apoptotic index in the combination treatment group was tested with Pearson's Correlation test. There was a fairly strong correlation between increased Endo-G expression and increased apoptosis index *(p<0.001, r=0.985)*

## DISCUSSION

Mitochondrial Endonuclease G is an enzyme encoded by the EndoG gene in humans.[14,15] Through DNA degradation, this protein participates in caspase-independent apoptosis when translocated under oxidative stress from mitochondria to the nucleus.[16] consequently, cancer cells will engage EndoG. Nuclear-encoded endonuclease is the protein encoded by this gene which is localized in the mitochondriallintermembranelspace.[14,17] EndoG will detach from mitochondria and move to the nucleus, then degrading chromatin with other nuclear proteins' help.[16,18,19] EndoG still binds to Hsp70 and CHIP under normal conditions. However, EndoG can dissociate Hsp70 and CHIP then translocate in the nucleus when experiencing oxidative stress which will cause apoptosis through DNA degradation. On the other hand, inhibitors of protein apoptosis (IAP) will be stimulated by EndoG to degrade proteasome by targeting proteins.[11,20] The lectin contained in Abelmoschus esculentus has been further studied for its anti-cancer effects. The results found that apoptosis can be induced by lectins starting from the interaction of lectin with sugar-binding lreceptorslonlthelplasmakmembrane so that endocytosis occurs. Lectin vesicles then migrate to mitochondria to produce reactive oxygen species (ROS), rip off the mitochondrial membrane and release EndoG into the cytoplasm. Thereafter, EndoG dissociates from Hsp70 and CHIP and transfer to the nucleus, degrading DNA to influence apoptosis. 15 days. The results found can reduce the diameter of the tumor and reduce the amount of density of breast cancer vascularization.

This study showed that Abelmoschus esculentus extract did not have efficacy alone given without chemotherapy as the main therapy. And when given in combination with chemotherapy, there will be a very high synergy effect. This can be caused because Abelmoschus esculentus can trigger apoptosis through the Caspase and Endonuclease-G pathways, where Endonuclease-G substances and other pro-apoptotic cytokines that trigger apoptosis from the Caspase pathway are abundant in the mitochondrial double layer membrane. If Chemotherapy is added, this chemotherapy agent, especially Adriamycin, will form free oxygen radicals, accelerating the breakdown of the mitochondrial double-layer membrane.[21-24] Thus, accelerating the triggering of apoptosis via the Endonuclease-G pathway.

The limitation of this study, we did not measure the many intermediate cytokines on the apoptosis cascade. It seems like BAD, BAK, IAP protein, and the role/ influence of Fas Associated Death Domain

## CONCLUSION

Abelmoschus esculentus extract can increase the apoptotic index against chemotherapy in vivo in rats through the Endo-G pathway when given in combination with AC chemotherapy

## ETHICAL APPROVAL

The animal experiment was approved by the Research and Ethics Committee of the Faculty of Medicine Diponegoro University, Indonesia (protocol number: 140/EC/H/FK-UNDIP/XII/2022).

## FUNDING INFORMATION

Nobody provided funding for this research project. The authors are responsible for all costs associated with this study

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## AUTHOR CONTRIBUTION DETAILS:

All authors have contributed equally to the preparation of this manuscript

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