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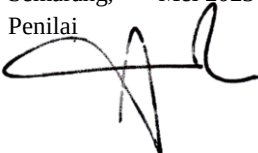
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(<https://balimedicaljournal.org/index.php/bmj/article/view/3835>)

Alfini Octavia, Wustha Farani, Syaina Azzahra, Safira Meita Hapsari

Online First: Feb 13, 2023 |

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Impact of *Abelmoschus esculentus* fruit extract on endonuclease_G and apoptosis index in adenocarcinoma mammae. A laboratory experiment: rats given chemotherapy AC
(<https://www.balimedicaljournal.org/index.php/bmj/article/view/4412>)

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 Abstract

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

by Selamat Budijitno

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

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 (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 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 adriamycin 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 isoquercetin and quercetin-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 allocation 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

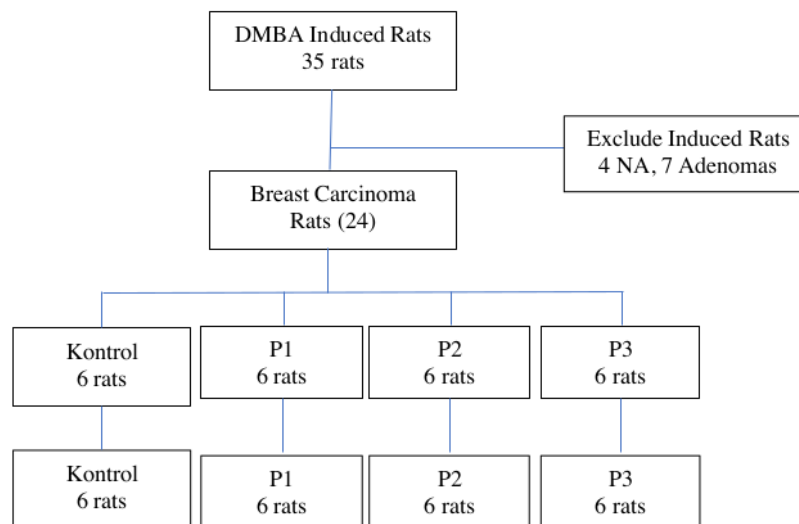


Figure-1. CONSORT diagram. Rats were randomly assigned to receive management either with Abelmoschus esculentus solution (treatment group) or with 0,9% NaCl (non-treatment group)

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 Homogeneity 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).

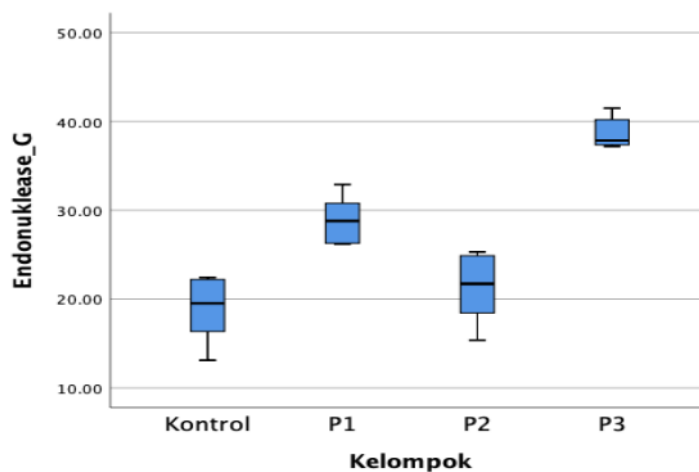


Figure-2, The Bonferroni post hoc test of Endonuclease-G Expression, (K vs P1, $p < 0.001$), (K vs P2, $p > 0.05$), (K vs P3, $p < 0.001$), (P1 vs P2, $p > 0.05$), (P1 vs P3, $p < 0.001$).

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 Homogeneity 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).

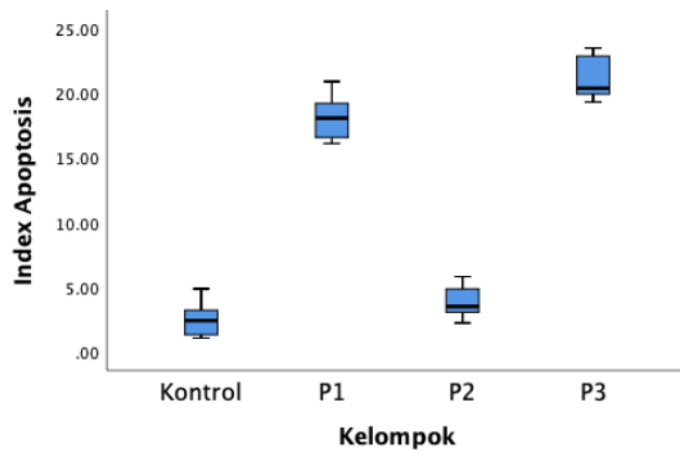


Figure-3, The Bonferroni post hoc test of Apoptotic Index, (K vs P1, $p < 0.001$), (K vs P2, $p > 0.05$), (K vs P3, $p < 0.001$), (P1 vs P2, $p > 0.05$), (P1 vs P3, $p = 0.028$).

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

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

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