TURNITIN-Impact-of-Abelmoschus-esculentus-fruit-extract

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

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ABSTRACT

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
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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, andlfluorouracil (CMF); and adriamycinlandlcyclophosphamide (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-testlonlylcontrol 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, the filtrate is taken, and 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 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

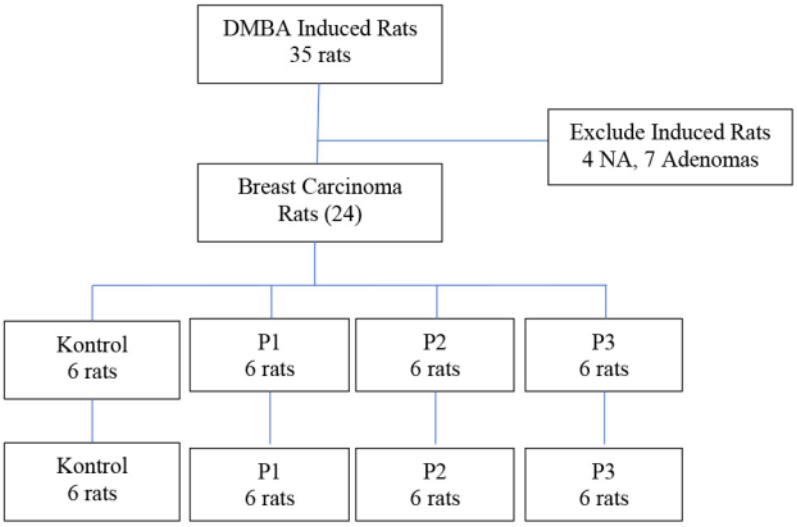

Figure 1. CONSORT diagram. Rats were randomly assigned to receive management either with Abelmoschus esculentus solution (treatment group) or with 0,9% NaCl (non-treatment group)

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	*P<0.001*
P2	21.2450±4.00108	
P3	38.6617±4.00108	

** = One-Way ANOVA*

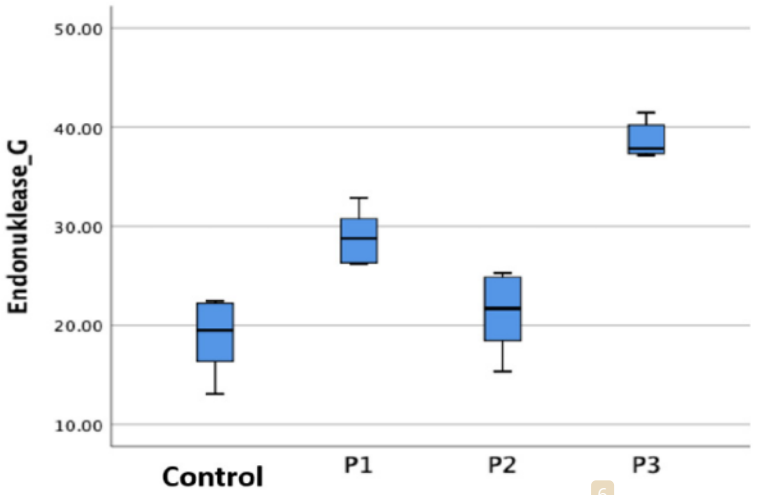

Figure 2. The Bonferroni post hoc test of Endonuclease-G Expression, (K vs. P1, p<0.001), (K vs. P2, p>0.05), (K vs. P3, p<0.001), (P1 vs. P2, p>0.05), (P1 vs P3, p<0.001).

Table 2. Apoptotic Index among Treatment and control groups

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

* = *One-Way ANOVA*

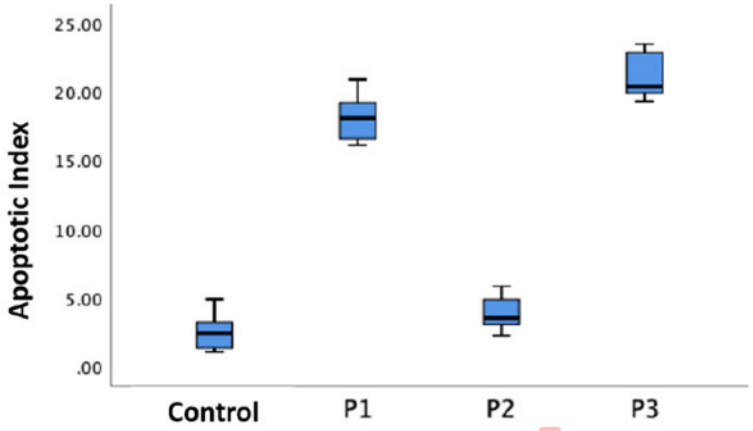

Figure 3. The Bonferroni post hoc test of Apoptotic Index, (K vs. P1, p<0.001), (K vs. P2, p>0.05), (K vs. P3, p<0.001), (P1 vs. P2, p>0.05), (P1 vs P3, p=0.028).

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**).

Groups P1 and P3 showed a greater Apoptotic Index than group K. There is no significant difference between Group K and P2. Groups P1 and P3 had a 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 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 dissociatesgfrom 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

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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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