The Effect of an *Annona muricata* Leaves Extract on Circulating Soluble Adhesion Molecules in Colon Carcinogenesis Model

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ABSTRACT

Background: *Annona muricata leaves are used as traditional tea drink that is currently being studied in the developing effort of treating some types of cancers because they are known as anti-cancer. Motivated by that usage, this study aims to analyse the potential of A. muricata leaves extract to be an anti-colon cancer by investigating the extract capability in reducing blood intercellular cell-adhesion molecule-1 (ICAM-1) and vascular cell-adhesion molecule-1 (VCAM-1).*

Method: *The research was conducted in Faculty of Medicine Universitas Indonesia with an experimental design. A. muricata leaves extract was tested in vivo. In vivo test was conducted to Swiss Webster mice induced with 10 mg/kg azoxymethane (AOM) and dextran sodium sulfate 1% (DSS). Statistical analysis used was SPSS.*

Results: *EIFAM reduce the serum level of ICAM-1 and VCAM-1. EIFAM significantly reduce serum level of VCAM compare to ESFAM.*

Conclusion: *Ethanol insoluble fraction of Annona muricata leaves water extract of is potential to be an anti-colon cancer proven by the extract capability to reduce ICAM-1 and VCAM-1.*

Keywords: *Annona muricata, ICAM-1, VCAM-1, colon carcinogenesis, in vivo*

ABSTRAK

Latar belakang: *Ekstrak daun Annona muricata digunakan sebagai minuman teh tradisional yang saat ini sedang dalam tahap uji terkait pengembangannya untuk pengobatan beberapa jenis kanker karena dikenal sebagai antikanker. Penelitian ini bertujuan untuk menganalisis potensi ekstrak daun A. muricata sebagai anti kanker kolon dengan menguji kemampuan ekstrak dalam menurunkan kadar intercellular cell-adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) dalam darah.*

Metode: *Penelitian dilakukan di Fakultas Kedokteran Universitas Indonesia dengan desain eksperimental. Ekstrak daun A. muricata diuji secara in vivo. Uji in vivo dilakukan pada mencit Swiss Webster yang diinduksi dengan 10 mg/kg azoxymethane (AOM) dan dextran sodium sulfate 1% (DSS). Analisis statistik yang digunakan adalah SPSS.*

Hasil: *EIFAM menurunkan kadar serum ICAM-1 dan VCAM-1. EIFAM secara signifikan mengurangi kadar serum VCAM dibandingkan dengan ESFAM.*

Simpulan: *Fraksi tidak larut etanol ekstrak air daun Annona muricata berpotensi sebagai anti kanker kolon yang dibuktikan dengan kemampuannya dalam menurunkan ICAM-1 dan VCAM-1.*

Kata kunci: *Annona muricata, ICAM-1, VCAM-1, karsinogenesis kolon, in vivo*

INTRODUCTION

Cancer is one of the world's biggest health care threats, with colorectal cancer (CRC) being one of the three most frequently encountered malignancy worldwide and the second most deadly cancer. The major cause of mortality associated with CRC is tumour invasion and metastasis. Although the deaths related to CRC are very high in high-income countries, the incidence and fatalities associated with CRC are also growing in developing countries.[1–2] The prevalence of colorectal cancer is escalating in Asia, where it is now the third most common malignant disease in both men and women. The International Agency for Research on Cancer (IARC) Global Cancer Observatory's GLOBOCAN 2018 database reported that the incidence of CRC in Indonesia was 30,017 out of 348,809 cancer cases, and it had the fifth-highest mortality rate (7.7%) among all cancers.[3–4]

The role of inflammation in most tumours remains unclear while it is emerging as one of the hallmarks of cancer. Long-term treatment with non-steroidal anti-inflammatory drugs is remarkably effective in reducing cancer rate and death, indicates that inflammation might have many as-yet-unrecognized facets. The various inflammatory processes underlying the development and progression of colorectal cancer. This means targeting anti-inflammatory means for its prevention and treatment.[5]

Intercellular adhesion molecule-1 (ICAM-1, CD54) displays an important role in the the pathogenesis of CRC. It is a cell surface glycoprotein of the immunoglobulin (Ig) superfamily and plays an essential role in cell-cell, cell-extracellular matrix interaction, cell signalling, and immune process. It is also expressed by tumour cells and modulates their functions, including apoptosis, cell motility, invasion, and angiogenesis. The interaction between ICAM-1 and its ligand may facilitate adhesion of tumour cells to the vascular endothelium and subsequently in the promotion of metastasis. ICAM-1 expression determines malignant potential of cancer.[6] VCAM-1 could be a suitable target for the development of anti-myeloma therapies.[7] The intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are two immunoglobulin superfamily adhesion molecules. Both are responsible to the endothelium adhesion of cancer cells and the immune responses of cancers.[8]

This study was motivated by empirically usage of *A. muricata* leaves as traditional tea drink that is currently being studied on the developing effort of treating some types of cancers. In vitro studies and cytotoxicity test in animal, also clinical trial have been conducted using the extract of these leaves.[9–10] This study aims to analyse the potential of *Annona muricata* leaves extract to be an anti-colon cancer by investigating the extract capability in reducing serum level of ICAM-1 and VCAM-1.

METHODS

Annona muricata L. extract

The *A. muricata* extract used in this study was the water extract and its fractions. They were ethanol-insoluble fraction (EIFAM) and ethanol-soluble fraction of *A. muricata* leaves water extract (ESFAM). Procedure of ethanolic fractination from water extract was as follow.

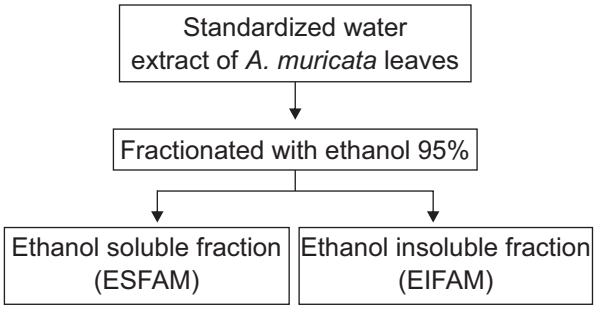

Figure 1. Procedure of fractionation on standardized water extract of *A. muricata* leaves

Animals

Experimental animals (n = 36) used were male Swiss Webster mice, aged 2–3 months, weighing 20–25 g. Mice were bred and reared in the Laboratory of Experimental Pathology, Department of Anatomic Pathology, Faculty of Medicine, Universitas Indonesia. Mice were kept in a room with temperature of 22 ± 2 °C in 12-hour light and 12-hour dark cycle. Mice were fed with standard pellet and *ad libitum* drinking water. Before being used in the experiment, the mice were confirmed free from parasitic diseases. 19 animals were reared and treated in accordance with the guide for the care and use of laboratory animals of the Animal Care and Use Committee, and had gained approval from the Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia (No. 0256/UN2. F1/ETIK/2018). Animal rearing and treatment were carried out by Felasa certified researchers.

Induction of Colon Carcinogenesis

Induction of colon carcinogenesis in mice was performed according to the method developed by Tanaka et al (2003) and Suzuki et al (2006). Mice were injected intraperitoneally with azoxymethane (AOM/sigma) dissolved in 0.9% natrium chloride (NaCl) at a dose of 10 mg/kg of body weight for one administration. Post-AOM induction the mice were given standard feed and mineral water for one week. For the next one week, the water was replaced with aquadest containing 1% dextran sodium sulfate (DSS/sigma). The mice were reared for first, second, third, and forth month until the time of sacrifice to get the colon tissue. The colonic tissue collected is the distal portion.

Extract Administration

Extract was administered orally to each mouse from the third week or after the completion of DSS administration. Mice were divided into 6 groups, each group consisting of 6 animals. The mice grouping were as follows: the first group or negative control group was the group of mice induced with AOM/DSS and only received distilled water during the treatment; the second group was mice induced with AOM/DSS and received 200 mg/kgBW of EIFAM (low dose); group 3 received 400 mg/kgBW of EIFAM (medium dose); while group 4 received 800 mg/kgBW of EIFAM per day (high dose); group 5 received 40mg/kgBW of ESFAM; and group 6 received 800 mg/kgBW of water extract per day. After six weeks of extract administration, the mice were sacrificed.

Blood samples were obtained from all colon carcinogenesis animals and were also obtained from healthy animal. Histological assessment was done in a blinded fashion to avoid bias. Our results indicate that multiple organ dysplasia was induced with azoxymethane (AOM) in male Swiss Webster mice (data has not been published). None of the mice showed to develop colorectal cancer.

Assay of soluble adhesion molecules levels of circulating ICAM-1 and VCAM-1 were measured with a commercial ELISA kit (MBS2701977, MyBioSource Inc).

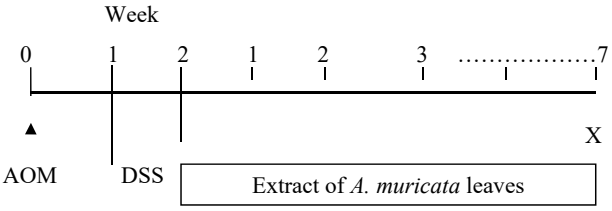

Figure 2. Research protocol (X, animals were sacrificed)

Data Analysis

Two-ways analysis of variance (ANOVA) was used to determine the effect of five doses of *Annona muricata* leaves extract (200, 400, 800 mg/kgBW of EIFAM and 40mg/kgBW of ESFAM; and 800 mg/kgBB of water extract per day). The Tukey multiple comparation test is used to determine the differences between groups. Prior to do ANOVA, the normality of data distribution was tested using the Levene's test, whereas the homogenity of variance was tested using the Kolmogorov–Smirnov test.

RESULTS

The animals' body weight were comparable in all groups at baseline, and all those variable was normally distributed ($p > 0.05$; Shapiro Wilk test). The between-group comparability analysis found that randomization ensured equal distribution of all variables (Table 1). All groups have an increase body weight throughout the eight weeks study, but there was no significant difference in the elevation between groups ($p = 0.469$) as shown in Figure 3.

Table 1. Animal body weight at baseline

Group	Average body weight (gram)	Between-group difference p-value
1	23.33	
2	23.00	
3	23.00	$p = 0.519$
4	22.00	
5	23.17	
6	22.57	

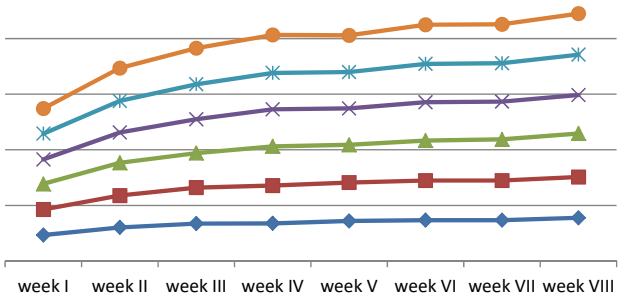

Figure 3. Animal body weight during experiment

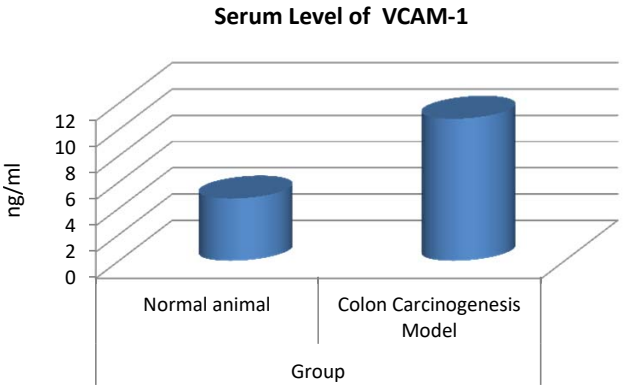

Figure 4. Serum levels of VCAM-1 in colon carcinogenesis animal vs healthy animal

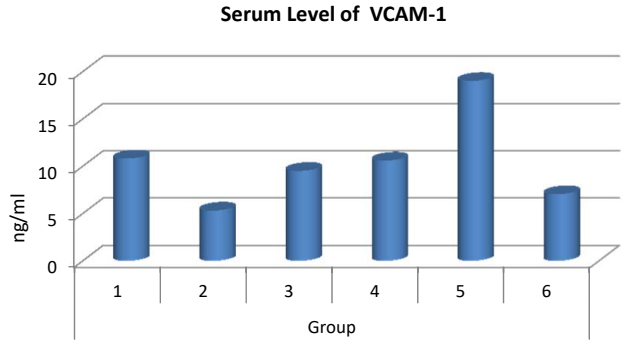

Figure 5. Serum levels of VCAM-1 after extract administration

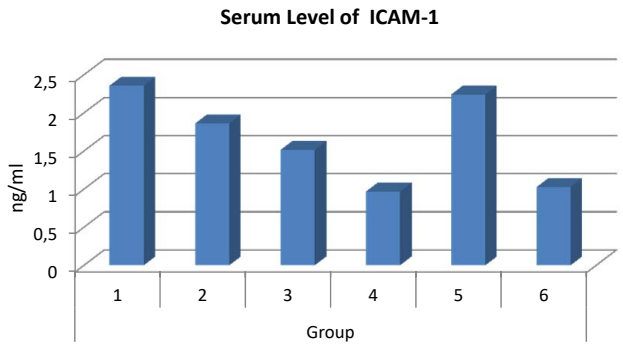

Figure 6. Serum levels of ICAM-1 after extract administration

The serum concentration of VCAM-1 was significantly elevated ($p < 0.01$) in colon carcinogenesis animal compared to heatlty animal as shown in Figure 4. Serum levels of VCAM-1 in extracts group (group 2 to 6) compared to non treated group (group 1) are shown in figure 5. Analysis using ANOVA method results $p = 0.000$ that means there was significant difference between group. Post hoc analysis reveals that differences exist between group 2 and 5 with $p = 0.000$. Serum levels of ICAM-1 tend to decrease in proportion to the extract doses as shown in figure 6, but there is no significant diffrence between groups.

DISCUSSION

Annona muricata L. is one of the many plant extracts that have been investigated owing to their anti-inflammatory and anticancer effects. The leaves of the plant have been extensively studied for its diverse pharmacological aspects and found prominent for anti-inflammatory and anticancer properties. However, most studies were based on crude extracts of the plant.[11]

In this study we used EIFAM as the main extracts with dose excalation. EIFAM reduced serum level both VCAM-1 and ICAM-1. EIFAM reduced significantly compare to ESFAM. This finding was in line with the result of previous study that EIFAM showed selective cytotoxic activities in vitro, while it contained the lowest concentration of flavonoid and annonacin.[12] A study has reported that soursop leaf ethanol extracts have twelve bioactive compounds profiles classified as alkaloid, flavonol glycoside, and monoterpenoid lactone derivatives, which have an antioxidant effect that scavenges the excessive reactive oxygen species (ROS). That study also indicated a therapeutic potential of *A. muricata* in kidney restoration in diabetes mellitus.[13] Another study investigated the effect of the aqueous extract of *A. muricata* leaves (AEAM) on TPA-induced ear inflammation and antioxidant capacity, showed its anti-inflammatory effect is associated with antioxidant capacity.[14]

Serum levels of VCAM-1 in extracts group (group 2–6) compared to non treated group (group 1) are shown in figure 5. Analysis using ANOVA method results $p = 0.000$ that means there was significant difference between group. Post hoc analysis reveals that differences exist between group 2 and 5 with $p = 0.000$.

The second group that received the lowest dose of EIFAM (200 mg/kgBW) has the greatest effect on VCAM reduction and showed a significant difference

with the fifth group that received ESFAM. This means EIFAM has a better effect on it compared to ESFAM, and the best effect is achieved with the lowest dose. If a compound effect is a function of dose, the effect of third (400 mg/kgBW of EIFAM) and fourth group (800 mg/kgBW of EIFAM) should be greater, but in this study on the contrary. It means this study does not depict the dose-response relationship. This could be because of the study period. Mice might be better investigated in one 10-week and one 20-week study to provide opportunity for the extracts to show further effects, especially the dose-response relationship. In others study using colon carcinogenesis model, trends in the results appeared to be highly dependent on sacrifice time-point.[15]

The effect of EIFAM on the reduction of serum levels of ICAM-1 tends to depict the dose-response relationship quantitatively. While neither VCAM nor ICAM seem not to decrease after mice were given with ESFAM (fifth group) compared to control group that only were given distilled water. Taken together with previous finding, EIFAM has better cytotoxic activities as well as anti-inflammatory activity.[9,10]

CONCLUSION

Ethanol-insoluble fraction of *Annona muricata* (EIFAM) leaves water extract is potential to be an anti-colon cancer proven by the extract capability to reduce ICAM-1 and VCAM-1. The effect of EIFAM in this study does not tend to be dose dependent. The limitation of this study could be the study period that limit the appearance of effect of the extract. This result also needs further investigation to ensure its effect and safety in human body, as well as to investigate the phytochemical content of EIFAM.

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