The effects of combination of *Eurycoma longifolia* Jack ethanolic extract and doxorubicine on hematological profile in rats given by 7,12-dimethylbenz(a)anthracene

L H Nurani¹, A Mursyidi¹, S Widyarini, A Rohman³

¹Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, Indonesia
²Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia
³Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia

E-mail: laelafarmasi@gmail.com

**Abstract.** Doxorubicin (Dox) is known as anticancer drug commonly used for cancer treatment. *Eurycoma longifolia* Jack or Pasakbumi was reported to have chemopreventive effect. In cancer patients, there are some dysfunctions of blood parameter, therefore some hematologic tests are needed to monitor cancer patients. In this study, the effects of combination of ethanolic extract of *E. longifolia* Jack (EE) and Dox on hematologic profiles were investigated in rats injected by DMBA. Rats were divided into eight groups. Group I was normal group; Group II, rats were treated with extract dose 100 mg/kgbw; Groups III, IV, V, VI, VII and VIII, rats were treated with Dox, DMBA, DMBA+Dox, DMBA+EE, DMBA+Dox +EE, and Dox+EE, respectively. DMBA administration orally was conducted twice a week for 5 weeks. At 16th week of treatments, bloods were taken from orbitalis sinus for hematologicals profile (levels of Hb, erytrocyte, hematocrite, leukocyte, MCV, MCH, and diferencial leucocyte count) measurements. These data were analyzed by one way ANOVA followed by LSD test. DMBA administration significantly decreased the hematological profiles compared to the normal group, except in lymphocyte level. Rats treated with extract and extract+Dox were able to increase the hematological profile compared to rats given by DMBA only. Based on these findings it can be concluded that the combination of EEE and Dox potentially increase hematological profile of rats given by DMBA.

**Keywords:** 7,12-Dimethylbenz(a)anthracene, doxorubicin, *Eurycoma longifolia* Jack, hematological profile

1. **Introduction**

*Eurycoma longifolia* Jack, known as Pasak Bumi in Indonesia and Tongkat Ali, in Malaysia is belong to Genus of Eurycoma and Family of Simaroubaceae. *E. longifolia* Jack is one of the popular tropical herbal plants widely used for the treatment of diseases. It indigenous to South-East Asian countries like Indonesia, Malaysia, Thailand and Vietnam [1]. The dried *E. longifolia* Jack root is available commercially with price between 20 and 25 US dollars/kg, and the water extracts are sold in higher price, i.e. 26 US dollars per bottle of 60 capsules [2]. *E. longifolia* Jack is also formulated as herbal tea, known as “*E. longifolia* Jack tea”
Some important pharmacological activities have been reported for *E. longifolia* Jack, namely antitumor and anticancer, antimalarial, anti-diabetic, aphrodisiac, anxiolytic and anti-parasitic activities, as reviewed by previous studies [2, 3]. In addition, *E. longifolia* Jack has been used to treat a variety of angiogenesis related diseases such as cancer, rheumatoid arthritis, obesity, and psoriasis [4, 5]. These activities are mainly due to the active components present in *E. longifolia* Jack. These components are including of quassinoid, alkaloids, glycosides, eurycomanol, and eurycomanone [6, 7]. Quassinoids are anticancer agents exhibiting a significant cytotoxic activity [8] and increases spermatogenesis [9].

Currently, the majority of health problems faced by human are cancer and the infectious diseases. The researchers and pharmaceutical industries have been trying to find out drugs either synthetic or herbal medicine to treat cancer [10]. In Indonesia, approximately of 8.2 million death is caused by cancers. It is estimated that the death due to cancer is 14–22 million for next 2 decades. Some dietary and medicinal plants, have been reported to possess substantial anticarcinogenic and antimutagenic effects to inhibit and treat the progression of cancer cells [11].

Some factors contribute to cancer, namely genetics, life styles, and carcinogens. One of chemical carcinogens belong to polycyclic aromatic hydrocarbon is 7,12-dimethylbenz(a)anthracene (DMBA) [12]. Doxorubicin (Dox) is known as anticancer drug commonly used for cancer treatment. Dox, however, revealed side effects toward normal cell. In order to reduce the side effects, doxorubicin is combined with plants extract, therefore the dose of Dox is reduced [13]. One of the potential plant used is *E. longifolia* Jack which has reported to have anticancer via anti-proliferative activity and apoptotic [14]. During chemotherapy, dysfunction of blood cell such as anemia [15] and thrombocytopenia [16] usually occurs. Therefore hematological profile test is needed. The aim of this study was to investigate the combination effects of *E. longifolia* Jack ethanolic extract and Dox on hematological profile.

2. Materials and Methods

2.1. Materials

The root of *E. longifolia* Jack was obtained from Martapura, South Kalimantan and the authentication was performed in Biology Laboratory, Universitas Ahmad Dahlan. The extraction procedure was conducted in the Laboratory of Pharmacy Biology, Universitas Ahmad Dahlan, Yogyakarta. The dried *E. longifolia* Jack was powdered and extracted using ethanol 70%. The 7,12-dimetilbenz(a)antracene (DMBA) was obtained from Sigma Aldrich (St. Louis, USA).

2.2. Animal treatment with DMBA, extract and doxorubicin

The animal treatment was performed according to previous study [17] using 56 female Sprague Dawley rats (60-70 g, 44 days old) which were purchased from Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Rats were randomly divided into 8 groups. Group I was normal group; Group II, which were treated with 100 mg/kg bw of EEE; Group III, which were treated with 1.12 mg/kg bw of Dox once a week for 5 weeks; Group IV, which were given by 20 mg/kg bw of DMBA orally, twice a week for 5 weeks. Groups V, VI, VII and VIII which were treated with DMBA + Dox, DMBA + ethanolic extract of *E. longifolia* Jack (EEE), DMBA + Dox +EEE, and Dox +EEE, respectively. The rats were weighted once a week. At 16th week of treatments, bloods were taken from orbitalis sinus for hematological profile (levels of Hb, erythrocyte, hematocrite, leukocyte, MCV, MCH, and differencial leucocyte count) measurements

2.3. Hematological profile tests

Hematological profile tests were carried out in Laboratory of Parahita Diagnostic Center (a Clinical Laboratory), Yogyakarta, Indonesia by determining the levels of hemoglobin (Hb), erythrocyte (RBC), hematocrite (HCT), leukocyte (WBC), MCV, MCH, MCHC, RDW, thrombosis (PLT), lymphocyte, neutrophil, eosinophil and basophil.
2.4. Statistical analysis
Hematological profile of each groups were analysed using one way ANOVA using SPSS versi 16 for Windows, after being tested for normality of data distribution and homogeneity of variances. The significance level was set at 0.05.

3. Results and Discussion
Hematological profiles of rat given by DMBA and treated with E. longifolia Jack ethanolic extract, doxorubicin and combination of both are presented in the Table 1 and Table 2.

Table 1. Hematological profiles of rats given by DMBA and treated with E. longifolia Jack ethanolic extract, doxorubicin and combination of E. longifolia Jack ethanolic extract and doxorubicin

| Group | Haemoglobin (g/dL) | Erythrocyte (million/µL) | Hematocite (%) | Leukocyte (µL) | MCV (fl) | MCH (pg) | MCHC (g/dL) |
|-------|--------------------|--------------------------|----------------|---------------|----------|----------|------------|
| Normal | 15.60±0.5 | 8.03±0.74 | 42.13±4.15 | 10162.50±3642.54 | 52.45±0.9 | 19.53±1.42 | 37.23±3.2 |
| EEE | 14.87±0.35 | 8.24±0.56 | 44.57±2.44 | 8796.67±1216.89 | 54.27±5.48 | 18.10±1.15 | 33.40±1.37 |
| Dox | 14.65±0.07 | 7.59±0.48 | 39.4±0.57 | 7045±1011.16 | 52.02±0.15 | 19.13±1.11 | 37.15±0.35 |
| DMBA | 14.72±1.29 | 7.40±1.09 | 41.88±4.11 | 8715.00±3902.35 | 57.27±6.91 | 20.05±2.97 | 34.95±1.36 |
| DMBA+Dox | 13.6±2.55 | 6.39±1.77 | 40.45±8.84 | 7120±254.56 | 63.75±8.89 | 21.55±2.05 | 33.75±1.06 |
| DMBA+E | 13.53±1.18 | 7.80±0.73 | 42.6±1.37 | 7962.50±1219.93 | 54.83±3.51 | 19.70±0.66 | 35.95±1.67 |
| DMBA+Dox +EEE | 13.3±0.38 | 6.77±0.49 | 39.00±2.34 | 7836.67±793.87 | 57.67±1.55 | 19.80±1.42 | 34.23±1.7 |
| Dox+EEE | 7.75±1.06 | 3.985±0.76 | 22.45±3.89 | 3595±1152.58 | 55.7±0.14 | 19.3±0.71 | 34.6±1.27 |

EEE, ethanolic extract of E. longifolia Jack; Dox, doxorubicin; DMBA, dimethyle benz(a)anthracene; RDW, Red blood cell distribution width; RBC, red blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin.

Table 2. Hematological profiles of rats given by DMBA and treated with E. longifolia Jack ethanolic extract, doxorubicin and combination of E. longifolia Jack ethanolic extract and doxorubicin

| Group | RDW (%) | Trombocyte (µL) | Lymphocyte (%) | Neutrophyle (%) | Eosinofil (%) | Basofyle (%) |
|-------|---------|----------------|---------------|----------------|--------------|-------------|
| Normal | 16.75±2.31 | 1159500.00±2455557.72 | 60.50±7.59 | 31.50±7.77 | 1.67±0.58 | 0 |
| EEE | 17.83±1.04 | 846000.00±345813.53 | 63.33±2.08 | 17.33±13.43 | 6.33±3.79 | 0 |
| Dox | 17.7±0.28 | 1215000±27092.5 | 68±0 | 26±0 | 2±0 | 0 |
| DMBA | 16.48±2.42 | 1145833.33±267947.6 | 63.20±7.66 | 28.60±5.64 | 1.60±0.55 | 0 |
| DMBA+Dox | 23.3±5.80 | 1121500±204353.86 | 60±0 | 36±0 | 3±0 | 0 |
| DMBA+ EEE | 16.45±2.42 | 667750.00±175049.28 | 64.00±5.66 | 22.00±4.24 | 5.00±1.41 | 0 |
| DMBA+Dox +EEE | 15.37±2.89 | 517333.33±516946.16 | 49.50±14.85 | 21.00±9.9 | 2.00±0 | 0 |
| Dox + EEE | 13.85±1.48 | 403000±125865.01 | 60±1.41 | 17±8.49 | 4.5±4.95 | 0 |

EEE, ethanolic extract of E. longifolia Jack; Dox, doxorubicin; DMBA, dimethyle benz(a)anthracene; RDW, red blood cell distribution width; RBC, red blood cell.

DMBA in the human body can cause oxidative metabolism of estrogencic hormones and result in some chemical metabolites such as reactive oxygen species (ROS) which capable to destroy the DNA. In addition, DMBA will attack hemoglobin to form haemoglobin adduct [18]. The hemoglobin adduct cause the level of haemoglobin reduce and, indeed, anaemia occurs. Anaemia can be identified by the reduced levels of MCV and MCH. Hemoglobin adduct is biomarker used to identify the presence of chemicals in the cell [19]. In the current study the rats given by DMBA revealed that the levels of hemoglobin, erythrocyte, hematocrite, leucocyte, MCHC, RDW, trombocyte, neutrophyle, eosinophile, basophil, MCV, MCH, and lymphocyte significantly lower than the normal group except...
for the leukocyte. The administration of EEE and Dox generally ameliorated the RDW, trombocyte, neutrophyle, eosinophyle, MCV, and MCH levels. In other hand, it decreased haemoglobin, erythrocytes, hematocrite, leucocyte, MCHC, and lymphocyte levels. These effects are attributed from the fact that haemoglobin causes oxidative reaction in haemoglobin. The E. longifolia Jack ethanolic extract can inhibit oxidative reaction via antioxidant mechanisms [20].

The erythrocyte levels of rats treated with EEE or Dox were not significantly different compared to DMBA group. However, the levels of erythrocyte in rats treated with EEE+ Dox reduced. Dox may reduce the interferon-γ (IFN-γ) production so that the erthropoesis and the formation of lymphocyte, monocyte, and neutrophil are inhibited [21]. The eosinophil levels in rats treated with DMBA were lower than those of normal groups, but it was not significantly difference (p>0.05).

4. Conclusion
The combination of E. longifolia Jack ethanolic extract and doxorubicin potentially increase hematological profile in rats given by DMBA.

Acknowledgement
The authors thank to the Ministry of Research and Higher Education of Republic Indonesia for financial support via competitive grant with contract number HB/III/2015.

References
[1] Khanam Z, Wen C S and HaqBhat I 2015 Phytochemical screening and antimicrobialactivity of root and stem extracts of wild Eurycoma longifolia Jack (Tongkat Ali) J. of King Saud Univ. Sci. 27 23–30.
[2] Bhat R and Karim A A 2010 Tongkat Ali (Eurycoma longifolia Jack): A review on its ethnobotany and pharmacological importance Fitoterapia 81 669–679.
[3] Rashid M, Kumar S and Ahmad B 2009 Medical Uses of Eurycoma longifolia Jack: a review Pharmaceut. Res. 2 70–78.
[4] Kavitha N, Noordin R and Chan K L 2012 In vitro anti-toxoplasma gondii activity of root extract/fractions of Eurycoma longifolia Jack BMC Complement. Altern. Med. 12 (1–8) 91.
[5] Al-Salahi A, O S, Kit-Lam C, Abdul Majid A M S, Al-Suede F S R, Saghir S A M, Abdullah W Z, Ahamed M B K and Yusoff N M 2013 Anti-angiogenic quassinoid-rich fraction from Eurycoma longifolia modulates endothelial cell function Microvascular Research 90 30–39.
[6] Bedir E, Abou-Gazar H, Ngwendson J N and Khan I A 2003 Eurycomaoside: a new quassinoid-type glycoside from the roots of Eurycoma longifolia Chem. Pharm. Bull. 51 1301–1303.
[7] Low B S, Choi S B, Wahab H A, Das P K and Chan K L 2013 Eurycomanone, the major quassinoid in Eurycoma longifolia root extract increases spermatogenesis by inhibiting the activity of phosphodiesterase and aromatase in steroidogenesis J. Ethnopharmacol. 149 (1) 201-7.
[8] Jiwajinda S, Santisopasri V, Murakami A, Kawanaka M, Kawanaka H, Monique G M, Eilas R, Balansard G and Ohigashi H 2002 In vitro anti-tumor promoting and anti-parasitic activities of the quassinoids from Eurycoma longifolia, a medicinal plant in Southeast Asia J. Ethnopharmacol. 82 55–58.
[9] Low B S, Das P K and Chan K L 2013 Standardized quassinoid-rich Eurycoma longifolia extract improved spermatogenesis and fertility in male rats via the hypothalamic-pituitary-gonadal axis J. Ethnopharmacol. 145 (3) 706-14.
[10] Ali Ahmed H E, Abdel-Salam H A and Shaker M A 2016 Synthesis, characterization, molecular modeling, and potential antimicrobial and anticancer activities of novel 2-aminoisoindoline-1, 3-dione derivatives Bioorganic Chemistry 66 1–11.
[11] Youssef K M, Ezzo A M, El-Sayed M I, Hazzaa A A, EL-Medany A H and Araf M 2016 Chemopreventive effects of curcuminalanologs in DMH-Induced colon cancer in albino rats model Future J. Pharmaceut. Sci. 1 57-72.
[12] Adventus B, Tiny E H 2013 Ekstrak metanol daun kelor menurunkan ekspresi BCL-2, TRAIL-R1, dan kadar caspase-3 jaringan kolon tikus yang diinduksi DMBA J. Kedokteran Brawijaya 27 4.

[13] Ang H H, Hitot S Y, Fukaya H and Takeya K 2002 Quassinoids from *Eurycoma longifolia* Phytocemistry 59 833-837.

[14] Mahfudh N and Azimahtol HLP 2008 Eurycomanone induces apoptosis through the up regulation of p53 in human cervical carcinoma cells J. Cancer Mol. 4 (4) 109-115.

[15] Dicato M, Plawny L and Diederrich M 2010 Anemia in cancer Annals of Oncology 21 (Supplement 7) vii167–vii172.

[16] Kuter D J 2015 Managing thrombocytopenia associated with cancer chemotherapy. Oncology J. 29 (4) 282-94.

[17] Jenie R I, and Meiyanto E 2007 Ko-kemoterapi ekstrak etanolik daun sambung nyawa (*Gynura procumbens* (Lour.) Merr.) dan Doxorubicin pada sel kanker payudara Majalah Farmasi Indonesia 18 (2) 81-87.

[18] Ogawa M, Oyamate, Isse T, Yamaquchi T, Murakami T, Endo Y and Kawamoto T 2006 Hemoglobin adducts as a marker of exposure to chemical substances, especially PRTR class I designated chemical substances J. Occup. Health 48 (5) 314-28.

[19] N’jai A U, Larsen M, Shia L, Jefcoate C R and Czuprynski C J 2010 Bone marrow lymphoid and myeloid progenitor cells are suppressed in 7,12-dimethylbenz(a)anthracene (DMBA) treated mice Toxicology 505341-505349.

[20] Rifkind, Joseph M, Joy G, Mohanty, and Enika N 2015 The pathophysiology of extracelluler hemoglobin associated with enhanced oxidative reactions Front. Physiol. 5 500.

[21] Zhang, Xiao-Yu, Li, Wen-Guang, Wu, Yong-Jie, and Gao and Ming-Tang 2005 Amelioration of doxorubicin-induced myocardial oxidative stress and immuno-suppression by grape seed proanthocyanidins in tumour bearing mice J. Pharm. Pharmacol. 57 (8) 10431.