Chemopreventive Activity of Kola (Cola Accuminata) Seed Ethanol Extract in Mice Induced by Cyclophosphamide

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Abstract. Cola accuminata or kola is one of the plants that have activity as anticancer. Previous research has shown that the content of hexane extract from Cola accuminata seeds can kill 100% of breast cancer and prostate cancer. The objective of this study was to demonstrate the presence of chemopreventive activity of ethanol extract of cola seeds in cyclophosphamide-induced test animals. The experiment was conducted using experimental method using healthy mice. The kola seeds were extracted macerated for 48 hours with ethanol solvent. On the first day, the test animal induced by cyclophosphamide with a dose of 50 mg/kgBW intraperitoneal. Ethanol extracts of kola seeds were administered for test animals at doses of 200, 400 and 800 mg/kgBW for 2 days then animal was killed and dissected, blood drawn for hematocrit and femoral bone examination for micronucleus examination. Based on result of research known ethanol extract of kola bean able to increase hematocrit value but not statistically significant and on micronucleus observations showed a statistically significant decrease in the amount of micronucleus (P<0.05) with the best activity seen at doses of 800 mg/kgBW. The results concluded that the extract ethanol kola seeds have chemopreventive activity at dose 800 mg/kgBW.

1. Introduction
Cancer is a major death threat for some people [1]. Therefore, in the world cancer is one of the biggest problems of public health both developed and developing countries. From Jemal research in 2008, the incidence of cancer in the United States in 2008 amounted to 1,437,180 inhabitants with a death rate of 565,650 inhabitants [2]. In addition, the World Health Organization (WHO) stated that in 2005, 7.6 million people worldwide died from cancer and 7.9 million in 2007 [3]. In Indonesia at 2005 the number of cancer patients reached 6% of the population [3], and based on Basic Health Research, cancer ranks 6th largest cause of death in Indonesia each year 100 new cases occur among 100,000 populations.

The magnitude of the prevalence of cancer each year and the cost of cancer treatment, it is necessary to do the primary prevention, early detection and treatment techniques with appropriate methods to reduce the incidence of cancer. During this time the methods used against cancer include surgery, radiation therapy and chemotherapy with or without drugs. However, the use of this method has not been able to effectively combat the occurrence of cancer due to the narrow security limit and low selectivity of anticancer drugs in chemotherapy drug [4]. In addition, the toxicity of chemotherapy agents is very high because it can inhibit the division of normal cells of rapid proliferation such as bone marrow, germinativum epithelium, gastrointestinal mucosa, hair follicles and lymphocytes [4]. Therefore, the need for alternative medicine from plants that are effective, specific and safe against cancer which can inhibit the proliferation of cancer cells and cure cancer. Compounds that can inhibit
cancer growth or reduce the possibility of regrowth of cancer cells called chemopreventive agents [5].

Previous research has suggested that some active ingredients from plants have activity as anticancer. One plant that has anticancer activity is kola seed (Cola acuminata). Cola acuminata is one of tropical plants originating from South Africa and spread to Europe and Asia, one of Indonesia. Traditionally the cola plant is used as a medicine for diarrhea, dysentery, nausea and cough. At Lowe Research in 2012 proves that hexane extract from kola seed (Cola acuminata) can kill 100% breast cancer and prostate cancer [6]. In addition, it has a cytotoxic effect on breast cancer cells [7]. And able to reduce the viability of LNCaP and DU145 with growth constraints (GI50) [8]. The activity of kola seeds as an anticancer occurs by inducing the occurrence of apoptosis in cancer cells [9]. Therefore, this study was conducted to prove the chemopreventive effect of the ethanol extract of kola seed (Cola acuminata) to the mice tested animal’s cyclophosphamide induced.

2. Methods

This research is a type of randomize block design study, in which some test animals are grouped into 5 groups, ie group 1 as negative control, which is given only cyclophosamide solution without ethanol extract of kola seeds. Group 2 as the solvent control, given 1% CMC solution without ethanol extract of kola seeds. Groups 3, 4 and 5 as the test group, given ethanol extract of kola seeds of 200, 400 and 800 mg/kgBW. This research was conducted in the laboratory of Pharmaceutical Biology Faculty of Pharmacy University of Muhammadiyah Purwokerto, research laboratory and integrated testing unit IV and clinical pathology laboratory of UGM medical faculty.

2.1.Materials

The materials used in this study were kola seeds obtained from LIPI Bogor Botanical Garden, 96% ethanol (Bratachem), aquades (Bratachem), giemsa, cyclophosphamide, methanol pro. analys., xylol, emersi oil [10] The tools used are rotary evaporator (IKA® Basic), analytical scales, a set of maceration tools, laboratory glassware (erlenmeyer, beaker, measuring cup, measuring flask, test tube, funnel glass), hematocrit centrifuge, surgical instrument, microscope, Polytube, hematocrit capiller.

2.2. Ethanol extraction of kola seed

Dry kola seed powder 200 grams was macerated for 48 hours with 96% ethanol solvent at 2.5L. The extraction was repeated until 96% clear ethanol solvent. The liquid extract was filtered using filter paper and evaporated with a rotary evaporator with a temperature of 50ºC [11].

2.3. Classification and treatment of animal testing group

Animals used are white male mice (Mus musculus) wistar strain 2-3 months old as many as 30 tails with 30-40 gram weight. Before used the animal acclimatized for one week. Mice as many as 30 tails and divided into 5 treatment groups each consisting of 6 mice with mice reserve 1 tail consisting of negative control group, which only given 50 cyclofosfamid solution 50mg/kgBW without containing ethanol extract of kola seeds [12]. The solvent control group, given a 1% Na-CMC solution without ethanol extract of kola seed. The treatment group 1, given ethanol extract of kola seeds was 200 mg/kg BW. The treatment group 2 given ethanol extract of kola seeds was 400 mg/kg BW and the treatment group given ethanol extract of kola seeds was 800mg/kg BW. Each group of mice was placed in a plastic enclosure that was different from the size of 50 x 30 cm with husk base, with room temperature 23-25 º C, 70-85% humidity and light set with 12-hour light 12-hour dark regulator [13]. Prior to treatment, the mice were fasted for 18 hours, but during the treatment all the mice were fed a drink of tap water each of which was administered ad-libitum.

2.4. Administration of ethanol extract kola seed

On the first day of the test animals was induced carcinogenesis cyclophosphamide with doses of 50 mg/kgBW intraperitoneal for the positive control group and the test group [14]. Test animals were given a 1% NaCMC suspension and ethanol extract of kola seed (Cola acuminata) at doses of 200, 400 and 800 mg/kgBW for 2 days orally to each test group. For the negative control group only 1%
Na-CMC suspension was given peroral for 2 days. Then the animal killed and dissected and blood is taken for hematocrit value determination of hematocrit micro method and femoral bone marrow for micronucleus examination.

2.5. Determination of hematocrit value

The blood sample contained in a tube containing an EDTA anticoagulant is fed into a microhematocrit tube roughly ¾ part of the contents of the capillary tube [15]. One end of the microhematocrit tube is covered with a cristaseal plug. Lid the ‘plate’ where the capillary tube is opened and place a tube of blood on the plate with the capillary end portion being blocked outside, then the ‘plate’ closed again. Then centrifuged at 2,000 rpm for 30 minutes. The result of hematocrit determination is read by taking into account the height of the column.

2.6. Micronucleus observation

Bone marrow of femur mice taken by spat containing 1 ml of physiological NaCl then centrifuged at 100 rpm for 10 min [16] The resulting supernatant is removed by using a dropper, while the precipitate is used as a cell preparation. The cell preparation is then made of smear on the glass of the object, then dried [17] After drying, the object glass that has been smeared bone marrow femur cell preparations soaked in methanol for 10 minutes. Then the glass object soaked in 50 ml of May-Gruenwald solution for 3 minutes. Glass objects immersed in 100 ml of May-Gruenwald buffer phosphate (1: 1 v / v) solution for 2 minutes. The glass object is then washed with phosphate buffer. After drying, the glass object is immersed in 70 ml of Giemsa-buffer phosphate solution (1:10 v / v) for 15 - 20 minutes. Then the glass object was washed with phosphate buffer and continued with aquadest. After drying, the object glass is immersed in 95% ethanol for 45 seconds and lastly immersed in xylol for 3 minutes. After that it is dried and observed under a microscope with magnification 10 x 40 with the help of immersion oil. Calculation of the number of micronucleus cells on the glass object counted 5 times in different places. Micronucleus cells are dark purple while normal cells are purple. After that, determine the percentage of the number of micronucleus cells.

3. Results and discussion

Kola seed powder that has been macerated for 48 hours and evaporated, extract obtained 75,58 grams and rendemen obtained that is 18,66% (qualified yield is ≥ 7,2%). The organoleptic results of kola seed extract (Cola acuminata) are reddish-brown, distinctive odor of extract and viscous liquid.

Testing of chemopreventive activity of ethanol extract of kola seeds was done by hematocrit value checking method using microhematocrit and micronucleus method used to see the effect of extract on cyclophosphamide induced micronucleus inhibition. Cyclophosphamide is one of the drugs that has very rapid toxicity in fast-growing cells such as bone marrow [18]. All blood cells are made in the bone marrow, thus damaging the bone marrow will decrease the hematocrit value. So with the decrease of hematocrit values in the blood and the formation of micronucleus can be used as parameters of the occurrence of cancer effects.
Figure 1. The hematocrit value of the mice's blood on anticancer test as chemopreventive

There was no difference between negative group (40.25), ethanolic extract of kola 200 (41.25), dose 400 (41.75), dose 800 (39.75) and CMC solvent 1% (41) which is not statistically significant p > 0.05. However, an increase in hematocrit values is compared with negative control and solvent control.

Figure 2. Average Number of Micronucleus on Preparations of Bone Marrow Apus

In Figure 2 there is a difference in the number of micronucleus among the five treatments that are statistically significant (p <0.05). Significant differences occurred between cyclophosphamide induction groups administering cyclophosphamide induction (14.5 micronucleus) with a 1% (3.25 micronucleus) CMC suspension, 200 (12 micronucleus) 400 kola ethanol extract, 400 (11.50 micronucleus), 800 (6.25 micronucleus). There was no significant difference in increasing the dose of extract but the best activity was seen at dose 800 mg / kgBW with the least amount of micronucleus among the extract treatments, it means that induction of cyclophosphamide can be inhibited its formation by ethanol extract of kola seed, thus can be stated ethanol extract kola seeds have kemopreventif activity.

The anti-cancer effect of being chemopreventive expressed by micronucleus inhibition and elevated hematocrit values is associated with the presence of flavonoid compounds contained in the ethanol extract of kola seeds. Flavonoida is a secondary metabolite that has the potential as an antioxidant so that it can reduce the radical activity of superoxide anion and hydroxyl which increase due to cyclophosphamide metabolism.
4. Conclusion
Based on the results obtained, it can be concluded that the extract of ethanol kola seed potency as kemopreventif agent based on observation of hematocrit and micronucleus with best activity at dose 800mg / kgBB.

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