

Effect of extract purple Ipomoea Batatas cultivar kawi mountain chronic inflammation in Wistar Rats with atherogenic diet

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Abstract. The potential biological functions of purple Ipomoea Batatas cultivar kawi mountain (IBCK). anthocyanin from purple Ipomoea Batatas cultivar Kawi mountain have long been described in traditional system of medicine. However, the protective effect of atherogenesis is still unknown. The present study attempted to investigate the protective effect of extract purple Ipomoea Batatas cultivar kawi mountain against foam cell and chronic inflammation in Wistar rats with atherogenic diet. The biochemical atherogenesis CD40L, NF κ B and histopathological foam cells changes were examined. Our results show that the pretreatment with extract purple Ipomoea Batatas kultivar kawi mountain (20mg/kgBW) orally revealed attenuation of cytokines activities of CD40L, NF κ B and decreased formation of foam cells in wistar rats with atherogenic diet.

Keyword: *Ipomoea batatas*, *anthocyanin*, *rats*, *chronic inflammation*

1. Introduction

Inflammation, which “is a complex set of interactions among soluble factors and cells that can arise in any tissue in response to traumatic, infectious, postischemic, toxic or autoimmune injury” (Mallat *et al.*, 2013) [1] appears to be involved at all stages of atherosclerosis. It is implicated in the formation of early fatty streaks, when the endothelium is activated and expresses chemokines, including monocyte chemoattractant protein (MCP)-1 and interleukin (IL)-8, and adhesion molecules, including intercellular adhesion molecule (ICAM)-1, vascular adhesion molecule (VCAM)-1, E and P-selectin, leading to monocyte/lymphocyte recruitment and infiltration into the subendothelium (Hansson *et al.*, 2005) [2].

The NF κ B pathway is one of the main signaling pathways activated in response to proinflammatory cytokines, including TNF-, IL-1, and IL-18, as well as following activation of the Toll-like receptors (TLR) by the pattern recognition of pathogen-associated molecular patterns (PAMPs). Activation of this pathway plays a central role in inflammation through the regulation of genes encoding pro-inflammatory cytokines, adhesion molecules, chemokines, growth factors, and inducible enzymes such as cyclooxygenase-2 (COX2) and inducible nitric oxide synthase (iNOS) (de Martin *et al.*, 2000) [3].

CD40 is widely expressed on several cell types including leukocytes and vascular cells. The CD40 receptor is activated following ligation with CD40L (CD154). A large variety of immunological and vascular cells express CD40 and/or CD40L (Schonbeck and Libby, 2001) [4]. The CD40/CD40L interactions induce tissue factor expression on macrophages and ECs and diminish thrombomodulin expression, favoring a local procoagulant and prothrombotic status (Aukrust *et al.*, 2004) [5]. CD40/CD40L expression is known to be up-regulated in atheroma-associated cells but the exact regulating mechanisms remain largely unknown. In vitro studies have shown that CD40/CD40L interactions on the EC surface result in endothelium and SMC activation and subsequent adhesion molecules expression, an initiating step in atherogenesis (Schonbeck *et al.*, 2002) [6].

It had been proposed by the beginning of the 1980s that lipid-laden foam cells can migrate back from the intima into the bloodstream by crossing the arterial endothelium (Gerrity, 1981) [7]. Yet the molecular mechanisms responsible for macrophage emigration from the atherosclerotic plaque were totally unknown. Many bioactive compounds and extracts from plants such as purple Ipomoea Batatas cultivar Kawi mountain have thus been investigated for inflammation and antioxidant effects against atherogenesis. Therefore, there is a great demand for development of an effective cardioprotective drug from the natural products. Traditional healers of different regions in Indonesia used purple Ipomoea Batatas species for treatment of various ailments. Purple Ipomoea batatas cultivar kawi mountain species is one of the richest resources of bioactive flavonoids in 12 subgroup kimia structure included: flavones, flavonols, flavanonols, isoflavones, anthocyanins, chalcones, anthosianidins, leucoanthosyanins, dihydrochalcones, aurones, dan catechins (Machlin, 1991) [8]. The published reports of various biological activities of Ipomoea batatas species include hyperglycemic, anti-inflammatory, antitumor, antilipid, antioxidant potential. Recently, studies from our laboratory showed protective effect of purple ipomoea batatas against chronic inflammation atherogenesis. However, several biological activities of purple Ipomoea batatas have been reported; there is no scientific evaluation available in support of the

cardioprotective activity of purple Ipomoea batatas cultivar kawi mountain. Kowalczyk research in 2003 result that anthocyanins inhibit inflammatory processes (Kowalczyk et al ., 2003) [9]. Gunstone study also said the same thing how the plants were able inhibition atherosclerosis (Gunstone et al., 2002) [10]. Suda also suggests that anthocyanins from Ipomoea Batatas cultivar ayamurasaki act as radical scavenger and antimutagenic (Suda, 2003) [11]. Tokusoglu also suggests that Turkish cultivars Ipomoea batatas also play a role in the process of atherosclerosis and antiosianinnya bioactive activity is determined by how to cook (Tokusoglu et al., 2012) [12].

2. Materials and Methods

2.1. Collection of Plant Material.

The fresh aerial parts of the Purple ipomoea batatas cultivar kawi mountain plant were collected from kawi mountain near Malang east Java ,Indonesia. The plant was authenticated at Botanical Survey of Indonesia, Malang. The voucher specimen of the plant has been retained in the Department of Biology, Brawijaya University, Malang. The samples were collected from Gunung Kawi, East Java with water content of 59-65%

2.2. Preparation of Extract.

The samples were macerated for 14 hours with 0,01% HCl in ethanol. The solvent were then evaporated at 45°C using rotary evaporator under vacuum pressure, leaving water from the samples behind. Crude extract were centrifuged at 4500 rom for 30 minutes, then the supernatant were divided into two. The first partition were freeze dried and showed crude extract yield of 2,79g/100g sample (fresh weight). The other half were then extracted from the water solvent to ethyl acetate. In this research a modified method were conducted by using modified flash column chromatography with polyamide CC-6 resin, using water and ethanol as eluent. The yield of anthocyanins was 0,132g/100g sample (fresh weight). The antioxidant capacity was measured using DPPH method, IC₅₀ and LCMS spectrum .

2.3. Animals.

Male Wista rats of body weights ranging from 130 to 180 g were obtained from central Animal house of Malang and housed in an air-conditioned room at 24± 2 °C and 65-70% relative humidity with a 12 h light-dark cycle. The protocol used in this study was approved by the Ethic committee for animal Experimentation of the Polytechnic Health Malang. Diets were prepared following American institute of Nutrition (AIN) modification recommendations. The animals were given water ad libitum during the experimental period. Percentage atherogenic diet: carbohydrate 34% energy, fat 50% energy, protein 16% energy. Percentage normal diet, carbohydrate 70% energy, fat 10% energy, protein 17% energy, other 3% energy.

2.4. Treatment Design.

Animals (Male Wistar rats) were randomized and divided into five groups (5) of five animals each. Group I control negative group with normal diet, group II control positive group with atherogenic diet, group III, IV, V atherogenic diet +extract purple ipomoea batatas cultivar kawi mountain with doses 5, 10, 20mg/kg BW.

2.5. Assessment of cardioprotective Activity

2.5.1. Biochemical Estimations.

On day 90 after atherogenic diet administration, blood samples were collected by direct cardiac puncture using light ether anesthesia. Serum was separated by centrifuging at 2500 rpm for 15 min , used for analysis of CD40L, NFκβ, and formation foam cells by using standard ELISA Kits .

2.5.2. Antioxidant Parameters.

The antioxidant capacity was measured using DPPH method, with IC₅₀ of crude extract and ethanol fraction as much as 34,43ppm and 5,62ppm respectively, which showed strong antioxidant activity. The LCMS spectrum of aglycon confirmed the presence of petunidin and cyanidin.

2.6. Histopathological Analysis.

A small portion of aorta fresh cryocut and staining with oil red O and look in the microscope Olympus, 400 times.

2.7. Statistical Analysis.

In the statistical calculation result looks Parametric Kolmogorov - Smirnov normality test looks CD40L, foam cells are normal. In a non-parametric test of Kolmogorov - Smirnov one sample test results are shown for NFκB is normal. Furthermore oneway ANOVA post hoc premises turkey and homogeneity test shows that for the parameters of CD40L, foam cells and Kruskal wallis test for parameter NFκβ.

3. Results

3.1. Effects of purple Ipomoea batatas cultivar kawi mountain extract on CD40L and NFκβ Levels.

The effects of purple Ipomoea Batatas cultivar kawi mountain on serum CD40L and NFκβ levels in atherogenesis animals diet were represented in Figure 1. The levels of CD40L, NFκβ in serum day 90 and foam cells were significantly decreased treated group (5mg, 10mg, 20mg/kgBW). After administration of extract Ipomoea batatas cultivar kawi mountain to inflammation atherogenic treated animals, the levels of CD40L and NFκβ were significantly CD40L $p < 0,05$, $p = 0.048$. In a non-parametric test used Kruskal-Wallis was significant for NFκB with $p < 0.05$, $p = 0,000$.

3.2. Effect of purple Ipomoea batatas cultivar kawi mountain on histopathology.

Histopathological analysis revealed, normal diet control group showed normal smc in aorta (fig. 4), and atherogenic diet control positive group formation foam cells smc in aorta (fig. 5). Treatment with purple Ipomoea batatas cultivar kawi extract showed decrease formation of foam cells in doses 5mg, 10mg, 20mg/kg BW.

CD40L analysis

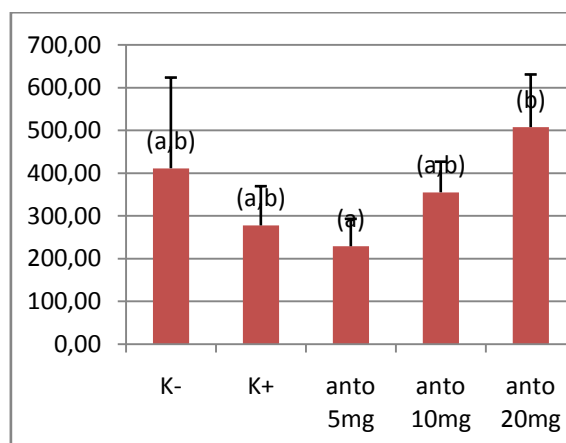


Figure 1. The level of CD40L was analyzed by ELISA kit. ICBK inhibit the CD40L at 5 mg/kgBW. Interestingly at highest doses 20mg ICBK could not inhibit CD40L.

NFκβ analysis

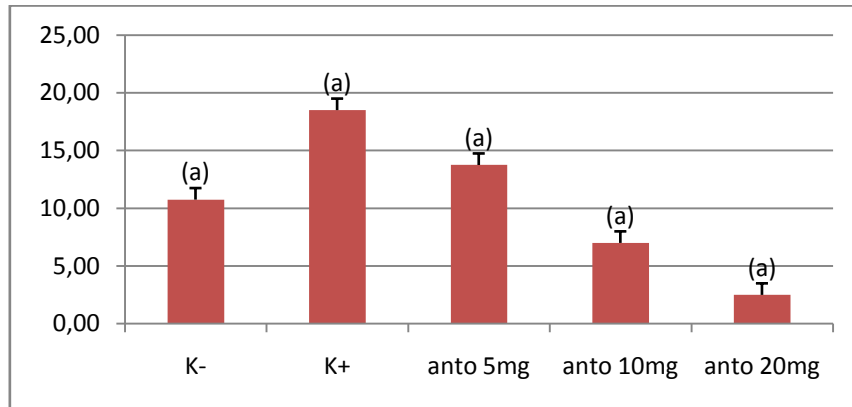


Figure 2.The level of NFκβ was analyzed by ELISA. ICBK inhibit NFκβ at dose 5mg/kg BW and highest inhibit in dose 20 mg/kg BW.

Foam cells analysis

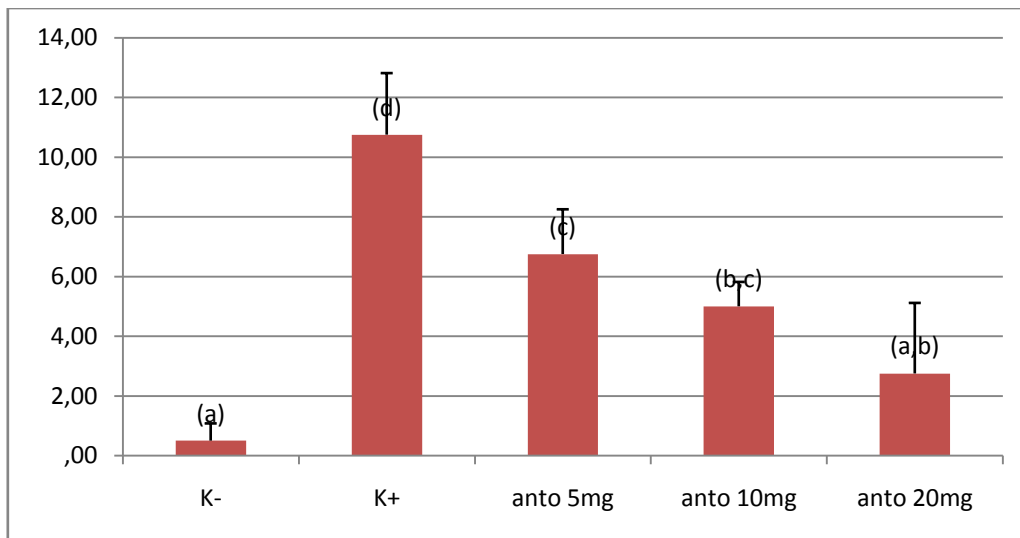


Figure 3. The level of foam cells was analyzed by histopathology. ICBK inhibit formation of foam cells at dose 5 mg/kg BW. In dose 20 mg/kgBW highest inhibit formation of foam cells

Foam cells in normal and atherogenic diet

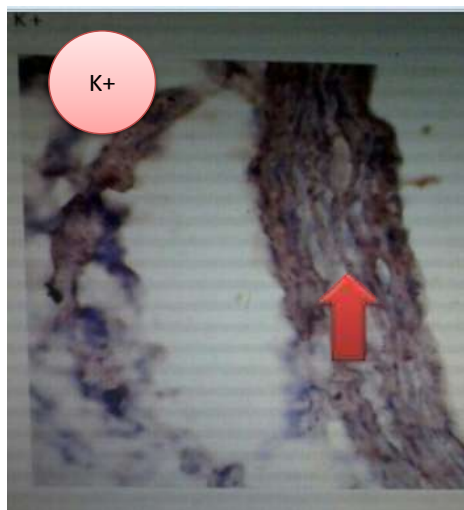


Fig.4 foam cell in kontrol positive SMC Aorta rat. Methods cryocut with staining oil red O (microscope olympus, Times 400x, 60 foam cell)

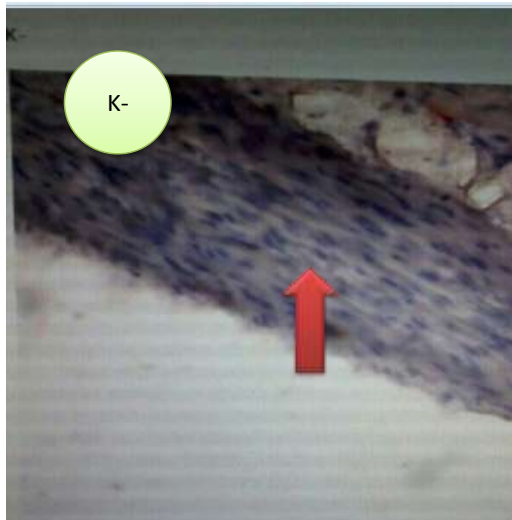


Fig .5 Negative control (normal diet) red code is aorta rat SMC with metods cryocut staining oil red O(Olympus microscope,times 400X, 2 foam cells)

Foam cell in aterogenik diet dan antosianin



Fig .6. Foam cells in SMC aorta rat with atherogenic diet and anthocyanin doses 5,10,20 mg/kg BW.Red code foam cells by cryocut with staining oil red O (microscope Olympus, times 400x, 31,28,11 foam cells)

4. Discussion

The level of CD40L was analyzed ELISA kit as seen in fig.1. IBCK inhibited CD40L in the doses 5,10,20 mg/kg BW.Effect IBCK was antiinflammation showed in 5mg/kg BW dose and other effect in doses 10mg and 20mg/kg BW. This research requires more doses diversity not only 3 doses only. In research Charalambos 2009, inhibition of CD40L can cause a reduction in the formation of atherosclerosis because CD40L is co stimulator of inflammation atherosclerosis.

NF κ B biomarkers caused more numerous in activating NF κ B . NF κ B levels are highest in rats fed atherogenic than normal feed so that the feed atherogenic . NF κ B at various dose levels 5, 10, 20 mg anthocyanin seen that the highest dose of 20mg of anthocyanins Ipomoea batatas turns Kawi mountain cultivars

showed the lowest levels of NFkB. NFkB P65 here seen in rat aortic tissue. NFkB is low due Ipomoea batatas cultivar anthocyanin Kawi mountain implies that the activation of inflammation triggered by the activation of NFkB can be suppressed by anthocyanins from Ipomoea Batatas Kawi mountain cultivars with high doses eg 20 mg . This study is consistent with research enriching Mallat 2006 that the process of atherogenesis is influenced by decreased levels of NFkB and Ludvig et al 2000 caiaipo able to reduce the inflammatory process. It is the same with that NFkB which turned out to have an impact on the levels of atherosclerosis can be reduced by administration of Ipomoea cultivars Batatas Kawi mountain .

In the foam cells seen a link between one group to another as evidenced by different notations each table bar. Sel highest visible foam in atherogenic conditions and decrease during the administration of anthocyanin in accordance with the increase in dose is 5mg , 10mg and 20mg , so the dose the largest is 20 mg was the lowest form foam cells . Normal diet showed only a few foam cells forming. This study showed that anthocyanins Ipomoea batatas cultivar Kawi mountain in various doses was able to suppress the formation of cell busa. This result is consistent with previous studies that have been conducted and the provision of Ipomea batatas cultivars anthocyanins mountain Kawi impact of the reduction in foam cell formation which is the fore runner of atherosclerotic. Result this study is consistent with research Kowalczyk in 2003 that anthocyanins inhibit inflammatory processes. Gunstone in 2002 study also said the same thing how the plants were able menginhibisi atherosclerotic. Suda et al 2003 also suggests that anthocyanins from Ipomoea cultivars Batatas Ayamurasaki act as radical scavenger and antimutagenic. In conclusion, extract of Ipomoea Batatas cultivar Kawi mountain possesses anti-inflammatory action due to an inhibitory effect CD40L,NFκβ,formation foam cells in SMC aorta Rat. In addition, the foam cells formation in the rat with atherogenic diet and ICBK was lower compared to atherogenic diet.

Conflict of Interests

There is no conflict of interests.

Acknowledgments

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