

# Anthocyanin Effect from Purple Ipomoea Batatas Decrease Formation CD40L, NFkB, and MDA in Inflammation Aterogenesis

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**Abstract** The theory of atherosclerosis based on the inflammation caused by endothelial dysfunction, lipid infiltration, free radicals and inflammatory processes immunologic. The activate NFkB and induces the formation of proteins that enhance the immune system in atherosclerosis progression. Prevention efforts done through drugs or fitofarmaka, one of the herbs that contain anthocyanins which act as antioxidants. Antioxidants are substances that are capable of slowing or preventing the oxidation process. Oxidizing conditions can cause damage to proteins and DNA, cancer, aging, and other diseases. Anthocyanins here act as a free radical scavenger or exogenous antioxidants. Giving anthocyanin will lower cholesterol and reduce the formation of CD40 and TRAF2 complex will further decrease NFkB activity and also decrease the release of proinflammatory cytokines MCP-1 and increases contractility aorta. Purple Ipomoea batatas contains anthocyanins in levels high enough that the 110mg-210mg/100mg, considering the cheap price is also easy to grow plant is expected that this plant can be fitofarmaka for atherosclerosis.

This research method using mice fed atherogenic diet for 65 days while the other group was given atherogenic diet along with dietary anthocyanins from purple cultivars Ipomoea varieties baatatas kawi mountain after examination of the complex CD40-TRAF-2 were formed, so that the activation of NFkB and MDA known effects of increased or decreased by the provision of anthocyanins from Ipomoea batatas purple varieties such.

The expected result is a decrease in the formation of complex CD40-TRAF-2, NFkB, and MDA as evidence those anthocyanins from Ipomoea batatas able to be inhibiting the inflammatory process in atherosclerosis

Keywords: *Atherosclerosis, an antioxidant, anthocyanin*

## 1. Introduction

Cardiovascular disorders preceded by the formation of atherosclerosis. It has been proven that atherosclerosis is an inflammatory process / chronic inflammation resulting inflammatory cells (Ross, 1999). There are four theories are currently believed to be the basis of the inflammatory process of atherosclerosis because of the free radicals, lipid infiltration, and endothelial cell dysfunction and immunological processes [1].

Theory of free radicals and the oxidation process of atherogenesis mechanism is expected to dominate a target mechanisms which can be intervened by providing antioxidants, although this is still a perdebatan. Inflammation triggered by many paths. NFkB pathway activates proinflammatory cytokine responses include TNF alpha, IL-1, IL-8, TLR-PAMP. Activation of this pathway is central to the inflammatory cycle that regulate coding genes and proinflammatory cytokines, adhesion molecules, chemokines, growth factors, and inducible enzymes such as cyclooxygenase-2 (COX2) and inducible nitric oxide synthase (iNOS) [2]. Proinflammatory cytokines include TNF alpha group, IL-1, IL-2, IL-6, gamma IL-12/IL-18/IFN, CD40/CD40L, osteopontin and MIF whereas anti-inflammatory cytokines IL-10, IL-13, TGF beta [3]. CD40-CD40L interaction is known to be co-stimulatory molecules to activate antigen-presenting cells (APC) [4]. Soluble CD40 is autocrine, paracrine, and endocrine actions, and could trigger further atherothrombotic formation mechanism. CD40/CD40L participate in the formation of atherosclerosis [5]. In some studies found that oxidative stress can be prevented by various types of food. Flavonoids of various food ingredients derived from plants have been widely studied, and is believed to be protective against oxidative stress [6][7]. Flavonoids contained in plant if it consumed routinely it can protect body from disease kardiovaskuler and several other chronic diseases [8][9]. Turns flavonoids can improve vascular endothelial function [10], its can reduce LDL sensitivity to the effects of free radicals [11] and its can be hypolipidemic, anti-inflammatory as well as good antioxidant [12]. Flavonoids are important is anthocyanin. Anthocyanins are secondary metabolites in a group of flavonoids. Anthocyanin or commonly abbreviated as ACN are plant pigments that are soluble in water and cause the blue, purple and red on the plant tissue. Usually appear as glycosides or asilglikosida, both a representation of the aglycone anthocyanidins. The most frequent types of anthocyanins found in nature is cyanidin (Cy), delphinidin (Dp), petunidin (Pt), peonidin (Pn), pelargonidin (Pg), and malvidin (Mv) [13].

Anthocyanins attract the attention of researchers because of its activity as an antioxidant, antimutagenic, protecting liver function and antihypertensive. Anthocyanins are antioxidants that are effective for inactivation of hydroxyl and peroxy radicals. Antioxidant activity of anthocyanin is affected by

hydroxylation on the ring fenoliknya . Anthocyanin with free hydroxy groups have binding activity in radical and the hydroxyl group is more than one , especially in the B ring would increase antioxidant activity [14].

Sweet potato ( *Ipomoea batatas* L. ) is a creeping plant that lives in all weathers , in the mountains or on the coast. Purple sweet potato tubers contained in Bali turned out to have a fairly high content of anthocyanins [15] , and it have been studied have an antioxidant effect in the blood and various organs in mice subjected to oxidative stress [16].

Another study by Andre Frolov and Hui in 2007 in the form of in vitro studies of endothelial cells with CD40 as an inducer of proinflammatory then given anthocyanin subsequently measured IL - 6 , IL - 8 , and MCP - 1 ( monocyte chemoattractant protein - 1 ) , CD40 receptor and NFkB by western blott . Results showed that the presence of anthocyanin menginhibisi and monocyte adhesion to TNF receptor inhibition combined ligation of CD40 that has been bonded with CD40L in TRAF - 2 recruitment to lipid rafts . Complex formation CD40/TRAF-2 trigger NF - κB activation which initiate gene - gene transcription of inflammatory cytokines including IL - 1 , IL - 8 , and MCP - 1 . To represent the overall anthocyanin treatment of atherosclerosis . So that anthocyanins can effectively reduce plaque aterosklerostik progressive in experimental animals and in humans with normal activity of the LDL receptor [17].

## 2. Material and method

### 2.1. *Ipomoea batatas*

#### 2.1.1. Characteristic of *Ipomoea batatas*

*Ipomoea batatas* is commonly called yams or sweet potatoes or " sweet potato " is supposedly derived from the American continent . Botanical and agricultural experts estimate the origin of sweet potato plants are New Zealand , Polynesia , and the central part of America . Sweet potatoes are spread throughout the world especially tropical temperate countries , estimated in the 16th century . The Spaniards credited sweet potato spread to Asia, especially the Philippines , Japan and Indonesia [18].

Systematics ( taxonomy ) plants , sweet potato plants are classified as follows [19] : Kingdom : Plantae, Division : Spermatophyta, Subdivision : Angiospermae, Class : Dicotyledonae, Order : Convolvulales, Family: Convolvulaceae, Genus : *Ipomoea*, species : *Ipomoea batatas* . Watercress is a close relative to the water , Sri Morning , Widosari , Watercress Forest , Tread Horse , Rincik Earth , Watercress Army, Tatarompetan , Hitang Root , Watercress Fur , Big Tatampayan , *Ipomoea horsfalliae* .

#### 2.1.2 . Types of Sweet Potatoes

Sweet potato as raw material in the manufacture of flour has quite a lot of diversity , which is composed of local species and some varieties [20]. Potato shape is usually round to oval with flat surfaces to uneven . Potato skins are white , yellow , purple or reddish purple , depending on the species ( varieties ) it. Potato flesh is white , yellow or orange a little purple [19]. According to [21], potato skins and meat contain carotenoid and anthocyanin pigments that determine the color .

#### Comparing of antochianin level in various cultivated purple variety of sweet potatoes [22].

Name	Content anthocyanin
MSU 01022-12	33,89/100g
purple sweet potato from Indonesia Kawi Mountain	110-220 mg/100g
Ayamurasaki/Yamagawamurasaki (Japan purple sweet potato)	300mg/ 100g
RIS 03063-05	510,80mg/100g
MSU 03028-10	560-590,8mg/100g

### 2.1.3. Sweet potatoes



**Figure 1:** Purple Sweet potatoes cultivated Ayamurasaki [23]

Ayamurasaki and Yamagawamurasaki, two purple sweet potato varieties from Japan, has been cultivated commercially in several areas in East Java with a potential yield of 15-20 t / ha. purple tuber flesh, only the intensity keunguannya still below both the introduced varieties.



**Figure 2.** Sweet potatoes Antin -1 [24]



**Figure 3:** MSU 03028-10 clones had levels of 560 mg/100 g tuber anthocyanin, higher than the Japanese purple sweet potato varieties and Yamagawamurasaki Ayamurasaki anthocyanin that levels less than 300 mg/100 g tuber [23].

Several kind of anthocianin and group substitution

Aurantidin		-H	-OH	-H	-OH	-OH	-OH	-OH
Cyanidin		-OH	-OH	-H	-OH	-OH	-H	-OH
Delphinidin		-OH	-OH	-OH	-OH	-OH	-H	-OH
Europidin		-OCH <sub>3</sub>	-OH	-OH	-OH	-OCH <sub>3</sub>	-H	-OH
Luteolinidin		-OH	-OH	-H	-H	-OH	-H	-OH
Peonidin		-H	-OH	-H	-OH	-OH	-H	-OH
Malvidin		-OCH <sub>3</sub>	-OH	-OCH <sub>3</sub>	-OH	-OH	-H	-OH
Peonidin		-OCH <sub>3</sub>	-OH	-H	-OH	-OH	-H	-OH
Petunidin		-OH	-OH	-OCH <sub>3</sub>	-OH	-OH	-H	-OH
Rosidin		-OCH <sub>3</sub>	-OH	-H	-OH	-OH	-H	-OCH <sub>3</sub>

According to [25] peonidin and sianidin ratio determines the color of a sweet potato which are divided into 2 groups, namely the class of red or peonidin and sianidin ratio > 1 medium blue or sianidin dominant group is the

ratio of peonidin and cyanidin < 1 [25]. the process of integration in the digestive tract of anthocyanins undergo a process of absorption, metabolism, distribution and excretion in the human body which turns into aglycone anthocyanins [26].

## **2.2. Antioksidan and anthocyanin**

### **2.2.1 Antioxidants**

Collector antioxidant free radical scavenger free radicals or through the formation of anti-oxides and radicals are less reactive to the dismutase way, recombination and catalyzes the reduction and transformation of a non-radical (such as SOD). The second way is by preventing the formation of free radicals with a metal ion binding agent (chelator) and reduce hydroperoxide to hydroxide is less reactive. Antioxidants primary role is to prevent the formation of new free radicals by breaking the chain reaction and turn it into a more stable product. Examples of primary antioxidant, is the enzyme superoxide dismutase (SOD), catalase, and glutathione dismutase. Secondary antioxidant function capture of radicals and prevent chain reaction [27].

#### **2.2.1.1 Natural antioxidants**

Natural antioxidants can not be specifically defined. According to [28], a natural antioxidant in foods can be derived from (a) the endogenous compound of one or more components of food, (b) the substance is formed from the reaction during processing, and (c) a food additive that is isolated from the source natural. Most of the natural antioxidant derived from plants. Some common sources of natural antioxidants derived from plants. Some common sources of natural antioxidants from plants are algae, cereals, cocoa products, citrus, herbs and spices, legumes, oily seeds, plant extracts, protein hydrolysates, resin, pepper, onion and garlic, and olive.

[29], an antioxidant component in nature has a chemical structure different, generally as amino acids, ascorbic acid, carotenoids, cinnamic acid, flavonoids, melanoidin, certain organic acids, reducing agents, peptide, phosphatides, polyphenols, tannins, and tocopherol. Natural antioxidant compounds classified as a component phenols, protein, nitrogen compounds, carotenoids, and other components such as vitamin C, ketones, and glycosides [30].

## **2.3 Anthocyanin**

Anthocyanins (from the Greek word combination: anthos = "flowers", and cyanos = "blue") are water-soluble pigments that are naturally present in many plant species [31]. Anthocyanin-producing plants is actually a protective mechanism against environmental stress factors include UV light, cold and drought. Anthocyanin seen in  $\lambda_{max}$  between 465 and 550 nm, as significant by UV absorption between 270 and 280 nm. Above 635 nm anthocyanin can be identified [32].

The Ability of anthocyanin antioxidants due to its high reactivity as hydrogen donors and the ability of polyphenols derived radical to stabilize and delocalize unpaired electrons, as well as the ability metal ions.

### **2.3.1 Bioavailabilitas anthocyanin**

Biological efficiency of anthocyanins depends on the absorption and metabolism, tissue distribution and excretion. In general, anthocyanins are rapidly absorbed and eliminated. Once ingested anthocyanin can be absorbed in the stomach and small intestine. Having broken into aglycone and sugar molecules by microflora in the digestive tract to the liver and brought into circulation flow of blood and urine, the study rats fed a diet containing high anthocyanin anthocyanin found throughout the digestive organs stomach sampai liver started 15 days after administration. In the brain itself whereof  $0.25 \pm 0.05$  nmol / g tissue. But the anthocyanin itself has a low bioavailabilitas so that when absorbed in the form of food just nM to  $1 \mu\text{M}$  with low concentrations were detected in the blood. Anthocyanin excretion with a low limit is 0.004% to 0.1% of the food intake [33].

Purple sweet potato tuber contains a high anthocyanin and causes changes in lipid profile and Jawi Budiasa such research in 2011 which showed a decrease in serum cholesterol absorption occurs due to the constraints of cholesterol and bile acids in the intestine [34].

Inflammatory cytokine signaling pathways in the form of atherosclerosis. There are many pathways that play a role such as NF $\kappa$ B, JNK/AP-1, JAK / STAT, Smads and TLR / MYD 88. NF $\kappa$ B pathway activates proinflammatory cytokine responses include TNF alpha, IL-1, IL-8, TLR-PAMP. Activation of this pathway is central to the inflammatory cycle that regulate coding genes and proinflammatory cytokines, adhesion molecules, chemokines, growth factors, and inducible enzymes such as cyclooxygenase-2 (COX2) and inducible nitric oxide synthase (iNOS) [2]. Proinflammatory signal transduction pathways / proaterogenik [3].

## 2.4. NFkB

NFkB is a dimeric transcription factor that shaped hetero or homodimerisation Rel family of proteins that includes p50 and p65 . This inactivate the formation of NFkB inhibitor that binds to kB ( I- kB ) in the cytoplasm . Proinflammatory cytokines are pathogenic formed by converging signals due to the activation of IKB kinase complex contains two kinases IKKI and IKK2 and NEMO regulator protein , activates IKK phosphorylation Ikb initiation at specific NH2 terminal serine residue . Ikb initiate phosphorylation of 26s proteasome degradation . NFkB spending dimer of the cytoplasmic NF - k - Ikb complex and translocation in the nucleus . In the nucleus binds to kB NFkB on specific genes and promote transcription . Target gene synthesis NFkB which includes Ikb $\alpha$  NFkB activated . Negative feedback regulation provides enhanced oscilasi on NFkB translocation [3].

Several studies have shown that antioxidants such as aspirin , flavonoids and N acetylcysteine inhibits activity NFkB . NFkB activity was also identified in the smooth muscle cells ( smooth muscle cells ) , macrophages and endothelial cells in human atherosclerotic lesions [35]. NFkB endothelial activation by reactive LDL showed very early occurrence in rats due to the high fat containing foods and the formation of atherosclerosis in the proximal aorta [36].

## 2.5. Inflammatory cytokines

Proinflammatory cytokines include TNF alpha group , IL - 1 , IL - 2 , IL - 6 , gamma IL-12/IL-18/IFN , CD40/CD40L , osteopontin and MIF whereas anti-inflammatory cytokines IL - 10 is IL-4/IL- 13 , TGF beta [3].

## 2.6. CD40/CD40L

CD 40 receptor activated after ligation with CD40L . CD 40 and CD40L found in human atherosclerotic plaques. CD40L expressed on the platelet membrane surface in the gap of a normal platelet membrane fragments called soluble CD 40L ( sCD40L ) [37].

Soluble CD40 is autocrine , paracrine , and endocrine actions , and could trigger further atherothrombotic formation mechanism . CD40/CD40L participate in the formation of atherosclerosis [5] .CD40 - CD40L interaction is known to be co stimulatory molecules to activate antigen - presenting cells ( APC ) and T cells contribute to change and B [4].

## 2.7. Dislipidemia

Composed of lipoprotein apoprotein components , cholesterol , cholesterol esters and triglycerides . Important first stage is the LDL is oxidized and then transferred by special scavenger receptor on macrophages [38]. Amount of fatty acids bound to the LDL molecule approximately half of whom are 2600 Poly Unsaturated Fatty Acid - PUFA ( polyunsaturated fatty acids ) such as arachidonic acid ( 12 % ) , linoleic acid ( 86 % ) dokosa hexanoic acid ( 2 % ) . PUFA is highly susceptible to oxidation due to carbon double bond but PUFA protected by lipophilic antioxidants that go beyond LDL [39].

Early atherosclerosis characterized by fatty streak lesions containing T cells and foam cells ( lipid - laden macrophages ) . Macrophages have fagositosis number and store excess lipids and cholesterol esters in the cytoplasm kolesteril . Foam cell formation involving bond - modified LDL with the LDL receptor on macrophages ( Sc - R ) . One modification of LDL occurs in vivo is oxidation , and is highly atherogenic . Dyslipidemia is influenced by pathological conditions such as diabetes , hyperthyroidism , nephrotik syndrome , CRF , Cushing's syndrome , etc. , are also drugs that lower LDL or raise HDL , estrogen , etc. cysclosporin . Lifestyle also determine such as smoking, alcohol , and stress [40].

## 3. Discuccion

Ipomoea batatas extract implications effect Kawi mountain cultivars against chronic inflammation provides a new idea that the anti-inflammatory ability can be used for preventive food daily in the prevention of atherosclerosis and also if this idea will continue to contribute to plaque rupture triggered inflammatory process.

## 4. Conclusion

Based on the results of existing research and discussion, we conclude :

- a. Extract of Mount Kawi cultivars Ipomoea Batatas known to contain powerful antioxidants

- b. Extract of Mount Kawi cultivars Ipomoea Batatas through this study demonstrate the ability as an anti-inflammatory by decreasing inflammatory biomarkers such as CD40L, NFkB, CRP
- c. The effect of anti-atherogenic also owned by Batatas cultivars Ipomoea Gunung Kawi visible from the ability to reduce the amount of foam cells in mice given atherogenic diet with various doses of the extract.

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