Effect of β-Carotene from Yellow Ambon Banana Peel on Rat Serum Retinol Level

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ABSTRACT

Vitamin A Deficiency (VAD) is associated with significant morbidity and mortality from common childhood infections and is the world’s leading preventable cause of childhood blindness. Studies showed that carotenoid is one of the promissed vitamin A sources. However the studies on carotenoid from yellow banana peel and its potential as a natural source of vitamin A has not been widely reported. This study was conducted to measure the blood serum retinol levels of rats after administration of β-carotene from yellow ambon banana peel. This was an experimental study with post test only control group design, with sample size of 18 rats with age 1 month, devided into 3 groups. The β-carotene dose administered based on the dose of red capsules vitamin A are (200,000 doses SI) for toddlers aged 12-59 months. Serum retinol levels were measured using a spectrophotometer according metide. This present study showed that the blood serum level in group treated with of β-carotene from yellow ambon banana peel (28.35 ± 1.61 mg/dL), was significantly different (p < 0.05) from that of control group (22.08 ± 1.35 mg/dL). The β-carotene from yellow ambon banana peel are potential as provitamin A.

Key word: β-carotene, retinol, peel, yellow ambon banana, VAD

INTRODUCTION

Vitamin A deficiency (VAD) is the leading cause of preventable blindness in children and increases the risk of disease and death from severe infections. [1, 2]. VAD especially among children continues to be a public health problem in Indonesia [3]. Therefore it is needed the effort to overcome VAD, one of the way to solve that problem is by giving them the food intake containing sufficient carotenoid or provitamin A.

Pro-vitamin A is seen as the red, orange, and yellow colored carotenes in fruits and vegetables. The most abundant and well-known carote is β-carotene. Provitamin A (beta carotene and other carotenes) must be converted in the body into retinol (vitamin A). Pre-formed vitamin A is already formed as vitamin A (retinol). Banana cultivars rich in provitamin A carotenoids may offer a potential food source for alleviating vitamin A deficiency [4, 5]. Yellow banana (Musa paradisiaca sapientum) has been widely consumed
as fresh fruit, processed, and used as industrial raw materials. The studies on carotenoid yellow banana peel and its potential as a natural source of vitamin A have not been widely reported.

The researchers of Taichung Chung San Medical Faculty Taiwan reported that banana peel extracted can avoid retina deficiency caused by light damage [6]. Suparmi and Prasetya [7] have done the research about the total carotenoid of yellow ambon banana peel was 6.203 ± 0.004 mg/g of vitamin A and carotenoids conversion was 124.06 ± 0.08 IU. Peel yellow banana (Musa parasidiaca sapientum L.) containing carotenoid pigments, among others zeaxanthin, xantofil, and β-carotene. The crude extract of carotenoid pigment of the yellow banana peel that has potentially as antioxidant with IC50 Value as 2350.3 ppm (higher than marker β-carotene with IC50 of 565.76 ppm) [8].

β-Carotene is vitamin A source and has the highest vitamin A activity among known carotenoid [9]. The best indicator to diagnose VAD is the measurement of serum/plasma retinol using High Performance Liquid Chromatography (HPLC) [3]. The content of retinol serum is indicator of biochemical vitamin A status as the determinant of the level of VAD in populations [10]. This study conducted to examine the activity of provitamin A of β-carotene pigment from banana peel in vivo to rat (Rattus norvegicus) Sprague Dawley with parameters levels of blood serum retinol. The results of this study are expected to contribute a banana peel processing technology, so can reduce the environmental pollution caused by the buildup of banana peel waste and reduce malnutrition in Indonesia, mainly the problem of VAD and blindness.

EXPERIMENT
Chemicals and instrumentation
The study was based on specimen of yellow banana peel collected randomly in from the banana traders around UNISSULA campus. The following chemicals were used including acetone (p.a-Merck), CaCO3 (Merck), ascorbic acid (Merck), filter paper, anhydrous Na2SO4 (Merck), N2 gas, gel silica Si-60, hexane (p.a-Merck). Male 18 rats, with age range 1-2 months, weight 200-300 g and was developed by the Animal Experiment Unit (UPHP) LPPT UGM. Ethical clearance was obtained from the Ethics Committee of the Medical Faculty Sultan Agung Islamic University.

Instrumentation applied for analysis in this study were rotary evaporator, column chromatography, UV-Vis spectrophotometer (UV-Beam Varian Cary).

Procedure reaction
Isolation and Purification of β-carotene from Banana Peel
Isolation of β-carotene from banana peel was done in Carotenoids and Antioxidant Research Center (CARC) Master of Biology Satya Wacana Christian University, Salatiga. Ambon banana peel samples of 200 grams grinded, then dissolved by acetone (p.a) with the sample and solvent ratio 1:10 w/v. At the extraction, added CaCO3 as a neutralizing agent and ascorbic acid as an antioxidant to prevent oxidation. The extraction was done in a dark room at a temperature of -15 °C to prevent oxidation or enzymatic degradation. Furthermore, the extract was filtered by filter paper, the residue obtained was extracted again with the same solvent until all the pigment taken up (banana peel becomes colorless). The extract is partitioned with hexane, and then filtered with the multilevel filter paper 42 in a cold state. Subsequently, the extract is added into anhydrous Na2SO4 to remove water content in the extract. Pigment extract resulted by filtration then was concentrated using a rotary evaporator.
Concentrated extract obtained is stored into the sample bottles and dried using N2 gas. Purification of carotenoid pigments by column chromatography using stationary phase silica gel Si-60 and a hexane mobile phase. Crude extract of the pigment dissolved in solvent hexane, then packed into chromatography columns that had been prepared. Each fraction collected in sample bottles and dried using nitrogen gas [11].

Animals Study

This was an experimental study with post test only control group design, with sample size of 18 rats with age 1 month, devided into 3 groups. Male 18 rats weighing ~100 g were obtained from LPPT UGM. Rats were kept in a temperature and humidity controlled room with a 12 h light-dark cycle. Water was provided ad libitum. The animals were allowed to acclimatize for at 1 week prior to the treatment.

The dose of β-carotene was based on the dose of vitamin A supplementation on the red capsule (200,000 IU) for toddlers aged 12-59 months. 1 IU is equivalent to 6 μg β-carotene, so β-carotene dose of the red capsule was 1,200,000 μg. The administration of β-carotene in rats applied a comparison table of the body surface area conversion dose of laboratory animal from human to rat (0.018).

The rats were divided into 3 groups of 6 rats each. Group I was a control group receiving no supplementation, group II was given β-carotene from yellow banana peel at the dose of 21.6 mg / rat / day, and group II was treated with pure β-carotene at the dose of 21.6 mg/ rat /day. The pigment was dissolved in cooking oil and given orally to rats as much as 3 mL / rat/ day using gavage for 14 days. On the day 15, blood samples were taken from the orbital sinus of the eye, then preparation of serum was performed. Serum retinol levels were evaluated using a spectrophotometer according metide [12]. The data on blood retinol levels were analyzed with one-way ANOVA.

RESULT AND DISCUSSION

The determination of vitamin A status is important to evaluate the levels of vitamin A in the body. Biologically, the function and histology, vitamin A status can be evaluated through the signs of xerophthalmia, night blindness, conjunctival impression cytology (CIC) and adjustments in the dark room [11]. Serum retinol level of rats treated with β-carotene from yellow ambon banana peel (26.35 ± 1.61 mg/dl) were higher than that of control rats (22.08 ± 1.35 mg / dl), but lower compared with rats treated with pure β-carotene (31.04 ± 2.13 mg / dl) (Figure 1).
One Way Anova test showed that there was a significant difference (p < 0.05) in the mean of retinol levels among treated groups. This suggests that β-carotene from banana peel banana has a potential as provitamin A. This might have been due to the conversion one of β-carotene molecule into two retinal molecules [9]. β-Carotene undergoes oxidative cleavage to retinal after entry. The retinal is then reduced to retinol, which is then esterified. Both retinol and retinal, essentially water-insoluble, are bound in the enterocyte to a member of the family of retinoid-binding proteins, cellular retinol-binding protein type I [CRBP(II)] [13]. The structure of β-carotene (provitamin A) and retinol (vitamin A) can be seen in Figure 2.

![Chemical structures of (a) β-carotene and (b) retinol](image)

**Figure 2.** The chemical structure of (a) β-carotene and (b) retinol [9]

Vitamin A (retinol) is a vitamin and is necessary for life and overall health. Vitamin A is especially important for the health of the eyes, lungs, bones, skin, immune system, and protein formation [14].

**CONCLUSION**

β-Carotene from yellow banana peel increases the blood serum retinol levels having potential as provitamin A.

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