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IPOMOEA BATATAS SYRUP DECREASE MALONDIALDEHYDE AND INCREASE NITROUS OXIDE PLASMA LEVELS AMONGST MODERATE SMOKER WORKERS AT DENPASAR

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Objective: Cells and tissues are continuously damaged by reactive oxygen species. Cigarette smoke is one of an exogenous source of free radical containing more than 4000 chemical compounds, that triggering the formation of free radicals related to diseases and aging process. Anthocyanins are potent antioxidants that are widely distributed in fruit, vegetables, red wines and Ipomea batatas. The aim of this study was to determine the effect of Ipomea batatas as a source of antioxidants in decreasing levels of malondialdehyde (MDA) and increasing of Nitrous oxide (nitrite/nitrate/NO_x) plasma in moderate smokers of workers at Denpasar. **Method**: This was an experimental study with a pretest-posttest control groups design. There are 33 moderate smokers who were divided into three groups, control group (placebo), treatment group with 15 ml purple sweet potato syrup (P1) and 30 ml (P2), for 14 days. All groups were performed the laboratory examinations for MDA and NOx plasma before and after the treatment. **Results**: This study showed that there were significant differences (p <0.05) both in MDA and NOx plasma levels in the control group, P1 and P2. The decrease of MDA levels on P1 was 35.39% and on P2 was 49.87%. The increase of NOx plasma levels was 7.78% for P1 and 14.68% for P2. **Conclusion:** From this study, it can be concluded that Ipomea batatas syrup contains of 8mg/mL anthocyanins, probably play a role in reducing the free radical and thus reducing the risk of disease and slowing the aging process.

Keywords: syrup purple sweet potato, anthocyanins, oxidative stress, moderate smoker

INTRODUCTION

Body cells and tissues are continuously damaged by free radicals and reactive oxygen species produced during normal oxygen metabolism or are induced by exogenous damage. Cigarette smoke is one sources of free radicals.[2,17] According to WHO in 2008, Indonesia is the third of 10 countries with the largest number of smokers in the world.[18] Cigarette smoke contains over 4,000 chemicals that can trigger the formation of free radicals in the body and causing oxidative stress.[22,25] Smoking has been strongly implicated as a risk factor for chronic obstructive pulmonary disease, cancer and atherosclerosis.

Cigarette exposure stimulates the formation of free radical in the body. The main target of free radicals is a protein, unsaturated fatty acids and lipoproteins, as well as DNA elements. Of the three targets, unsaturated fatty acids are the most vulnerable attacked by free radicals. A free radical takes an electron from the lipids cell membrane, initiating free radical attack on cells known as lipid peroxydation. Lipid peroxydation is a chain reaction initiated by free radical attack on phospholipids and polyunsaturated fatty acids in cellular membranes or sub cellular organelles. This resulted in the generation of complex aldehydes, ketones and the polymerization product with react and destroy the biomolecules, enzymes and nucleic acids. This condition will lead to diseases including aging. MDA is the most frequently biomarker for lipid peroxidation.[23] This is supported by a study, that rats exposure to cigarette smoke showed higher levels of MDA compare to rats non-cigarette exposed.[12]

Cigarette smoke contains superoxide and other reactive compounds. This smoke generates free radicals and ROS such as hydroxyl ($OH^{\bullet}$), peroxyl ($ROO^{\bullet}$) and superoxide ($O_2^{\bullet-}$) radicals.[7] Reactive free radicals that are generated from the gas and tar phase in cigarettes cause oxidative stress, increase lipid peroxidation and disrupt the antioxidant defense system.[21,24] An imbalance between antioxidants and oxidants potentially leading to oxidative stress, has been associated

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with the pathogenesis of many diseases, including cancer, aging, atherosclerosis, ischemic injury, inflammation and degenerative diseases.[3,21]

Smoking is a major risk factor of many diseases associated with aging, including Chronic Obstructive Pulmonary Disease (COPD), atherosclerosis and cardiovascular dysfunction.[13] Cigarette smoke is known to contain free radicals that can lead to decreased activity of nitric oxide directly or indirectly.[27] As shown in a study comparing 12 male smokers with non-smokers, it was found that in those who smoke found decreased levels of nitric oxide.[19] Nitric oxide is an endothelial-derived relaxing factor (EDRF) synthesized and released by endothelial cells and is a potent vasodilator. Decreased activity of nitric oxide as an endogenous vasodilator contributing to the low vascular resistance in the pulmonary circulation and can cause endothelial dysfunction.[14] A study, showed that after smoking cigarettes can significantly reduce levels of nitrite and nitrate, which is the end result of the metabolic nitric oxide. A low concentration of nitric oxide in the plasma, allowing the endothelial dysfunction, was found in long-time smokers. These conditions can accelerate coronary artery insufficiency and vasoconstriction in various tissues.[14]

Half-life of nitric oxide is very short in the tissue (about 3-4 seconds), therefore, it was difficult to examine directly. Indirect method was performed to observe the levels of nitrite/nitrate. Nitrite and nitrate (NO_x) are end result of nitric oxide metabolic. Significant decrease of nitrite/nitrate (NO_x) were seen in the plasma of smokers.[19]

Accumulated oxidative damage leads to a pathological process, and it can be prevented by antioxidants compounds.[7] The body needs antioxidants to neutralize free radicals. The major use of antioxidants is to destroy the chain reaction of free radicals in the body.[2] Flavonoids is a non-enzimatic antioxidant, and anthocyanins is one of them. An important effect of flavonoid in the scavenging of free radicals.[17] Previous study stated that flavonoids and other polyphenols significantly contribute to the total antioxidant activity of the body. Flavonoid consumption may prolong life by reducing inflammation and contribute to the reduction in coronary heart disease.[8] Anthocyanins belong to the class of flavonoids which are found mainly in the fruits and vegetables such as blueberries, elderberries, strawberries, blackberries, cranberries, raspberries, cherries, raisins, plums and sweet potato.[4]

Some root crops which commonly consumed by local communities, particularly in Bali, has been studied for their anthocyanin content. One of them is Ipomea batatas. In general, tubers of Ipomea batatas, contain higher anthocyanins than the other tubers. Ipomea batatas tubers contain anthocyanins varied between 110 mg / 100 g to 210 mg/100 g fresh weight.[26]

Research on the Ipomea batatas has been carried out in mice. The results showed that administration of Ipomea batatas extract could reduce levels of plasma MDA in mice with oxidative stress.[11] Not too many studies carried out regardless of the Ipomea batatas as a source of anthocyanins and its relation to free radicals, especially in smokers. Therefore, this study was carried out to assess the effect of purple sweet potato syrup as a source of anthocyanins in decreasing the MDA plasma levels in moderate smokers at Denpasar Bali.

MATERIALS AND METHOD

Subject

The subjects of this study were moderate smokers (smoking as much as 10-19 cigarettes per day), males aged between 20-50 years. No participant had a story of hypertension, diabetes mellitus or any other systemic disease. All participants gave written informed consent. This study has also approved of ethic by Sanglah Genaral Hospital/Faculty of Medicine Udayana University Ethic Commission.

The participants recruited in this study were 33 moderate smokers, divided into 3 groups: control group, treatment group with 15 mL Ipomea batatas syrup per day (P1) and treatment group with 30 ml Ipomea batatas syrup (P2). In the control group, placebo in the form of syrup that has a color similar Ipomea batatas syrup was provided. Intake of syrup treatment was carried out for two weeks. A double data checks was carried out, i.e a pre-test data before administering the syrup, and a post-test data after the administration of the syrup. MDA was measured using the established thiobarbituric acid (TBARS) method. Plasma NO_x levels, total nitrite and nitrate plasma levels were measured using the Griess Colorimetric methods of examination kits brands Assays design and reading the results using ELISA reader.

Preparation of Ipomea batatas syrup

Ipomea batatas syrup was made with the following procedure. Fresh Ipomea batatas were peeled and washed thoroughly and then were cut into pieces with a size of 2x2x2 cm. Pieces of Ipomea batatas were steamed for 1 hour until soft, then were cooled and placed in a fermentation boxes. Fermentation was carried out by adding 10 g commercial yeast (*Saccharomyces serevisae*) and incubated for 4 days at 27^{o}C. One kilogram of fermented sweet potatoes was then blended in 2 liter drinking water and filtered with three layers of cheese cloth. The filtrate was then added with sucrose and boiled for 45 minutes. The final sugar content of the syrup was 47.5%, while the anthocyanins content was 800 mg per 100 mL.[11]

RESULTS

The data of subjects characteristic, MDA and NO_x plasma levels were analyzed by the *Shapiro Wilk* to test the distribution and data normality, and homogeneity test with the *Levene test*. It was found that for the pre and post-test within each group were normally distributed and homogeneous (p > 0.05).

Comparability test of subject characteristics between groups using *One Way Anova test*. Based on the analysis by *One Way ANOVA test* showed that there was no significant difference (p> 0.05) in subject characteristic between groups, as shown in Table 1. Comparison tests before and after treatment between groups was done using *One Way ANOVA test*. The mean MDA plasma levels of the control group was 6.90±0.64, the group of treatment with 15 ml purple sweet potato syrup (P1) was 6.83±0.70, and the group of treatment with 30 ml purple sweet potato syrup (P2) was 7.16±0.55. Pre-test comparisons between groups using *One Way ANOVA* showed that there was no significant difference in MDA plasma levels between the control group, treatment group with 15 ml purple sweet potato syrup (P1) and treatment group with 30 ml purple sweet potato syrup (P2), (p> 0.05) as shown in Table 2.

Comparison test after the treatment of purple sweet potato syrup between groups as presented in Table 3. The mean of MDA plasma levels of control group was 7.60±0.45, the mean MDA plasma levels of treatment group with 15 ml purple sweet potato syrup (P1) was 4.91±0.61, and the mean MDA plasma levels of treatment group with 30 mL purple sweet potato syrup (P2) was 3.81±0.57. Post-test comparisons between groups showed that there were significant differences in MDA plasma levels between the control group with the treatment group with 15 mL purple sweet potato syrup (P1), between control with treatment group with 30 ml purple sweet potato syrup (P2), and also between treatment group with 15 mL purple sweet potato syrup (P1) and group with 30 mL purple sweet potato syrup (P2). The changes in MDA plasma levels were significant after the treatment with purple sweet potato syrup for 2 weeks (p <0.05). The decrease of MDA levels on P1 was 35.39% and on P2 was 49.87%.

The comparison test of NO_x plasma levels before being given treatment between groups was using *One Way Anova* test. The mean NOx plasma level of the control group was 2456.73 ± 137.41, the treatment group with 15 ml purple sweet potato syrup (P1) was 2486.64 ± 87.61 and the treatment group with 30 mL purple sweet potato syrup (P2) was 2391.73 ± 160.59. The pre-test comparisons between groups with *One Way ANOVA* showed that there was no significant difference in NO_x plasma levels between the control group, treatment group with Ipomea batatas syrup dose of 15 mL and 30 mL (p>0.05). As shown in Table 4.

Comparison test after intake of Ipomea batatas syrup between groups was using *One Way Anova*. The mean of NO_x plasma levels of control group was 2095.91 ± 281.98, and the mean of NO_x plasma levels of treatment group with 15 mL Ipomea batatas syrup (P1) was 2259.00 ± 119.99, and the mean of NO_x plasma levels of treatment group with 30 mL Ipomea batatas syrup (P2) was 2403.64 ± 207.23. Post-test comparisons between the groups with *One Way ANOVA* showed that there were significant differences in changes in NO_x levels between the control group, treatment group with 15 mL Ipomea batatas syrup, between control and treatment groups with 30 mL Ipomea batatas, and also treatment group purple sweet potato syrup dose of 15 mL and 30 mL. There is significant differences in the three groups after the treatment of Ipomea batatas syrup for 2 weeks (p <0.05). Based on the result an increase of NO_x plasma levels was 7.78% for treatment with 15 mL Ipomea batatas syrup and 14.68% for treatment with 30 mL purple sweet potato syrup. As shown as in Table 5.

DISCUSSION

Based on the results of this study, the MDA plasma levels of the moderate smokers who consumed Ipomea batatas syrup, 15 mL and 30 mL twice a day for 2 weeks significantly decreased. As well as the MDA, the NO_x plasma levels of the moderate smokers who consumed Ipomea batatas syrup, 15 mL and 30 mL twice a day for 2 weeks significantly increased. The purple sweet potato contains a high level of anthocyanin which is similar to bilberry. Anthocyanins from berries may have an effect on various physiological responses, including endothelial function[15]. Purple sweet potato tubers contain anthocyanin varied between 110 mg / 100 g to 210 mg/100 g fresh tuber.[26]

Cigarette exposure can trigger the formation of free radical in the body. The free radicals attack the unsaturated fatty acids, takes an electron from the lipids cell membrane, initiating free radical attack on cells known as lipid peroxidation.[12,23] Cigarette smoke contains superoxide and other reactive compounds, which are responsible for elevating levels of MDA as an end product of lipid peroxidation.

Cigarette smoke contains superoxide and other reactive compounds, can cause a state of oxidative stress and reduced endothelial nitric oxide. Cigarette smoke may decrease the activity of nitric oxide directly and indirectly. Smoking lowers the production of nitric oxide by lower levels of BH4.[6] Free radicals as well as superoxide contained in cigarette smoke, can affect endothelial and react with L-arginine. It may result in reduced

Table 1

Test Comparability of Subject Characteristics between Groups Before Treatment

Variable	Control	Ipomea batatas Syrup		*p*
		15 mL (P1)	30 mL (P2)	
Age	31.82±7.32	31.36±8.67	35.09±11.16	0.589
Duration of smoke	12.54±5.01	12.36±6.30	12.27±5.14	0.993
Number of cigarettes	11.82±1.25	12.27±1.42	11.90±1.14	0.679

Table 2
The Mean of MDA Plasma Levels between Groups Before Treatment of Ipomea batatas Syrup

Subject Groups	**N**	**Mean Levels of MDA**	**SD**	***F***	*p*
Control	11	6.90	0.64	0.809	0.455
P1	11	6.83	0.70		
P2	11	7.16	0.55		

P1= treatment with 15 mL syrup ; P2 = treatment with 30 mL syrup; SD = Standard deviation

Table 3
The mean of MDA plasma levels between groups after treatment of Ipomea batatas syrup

Subject Groups	N	Mean Levels of MDA	SD	F	*p*
Control	11	7.60	0.45	139.11	0.001
P1	11	4.91	0.61		
P2	11	3.81	0.57		

P1= treatment with 15 mL syrup ; P2 = treatment with 30 mL syrup; SD = Standard deviation

Table 4
The Mean of NOx Plasma Level Between Groups Before treatment of Ipomea batatas Syrup

Subject Groups	N	Mean Levels of NOx	SD	*F*	*p*
Control	11	2456.73	137.41	1.48	0.243
P1	11	2486.64	87.61		
P2	11	2391.73	160.59		

P1= treatment with 15 mL syrup ; P2 = treatment with 30 mL syrup; SD = Standard deviation

Table 5
The Mean of NOx Plasma Levels Between groups After Treatment of Ipomea batatas Syrup

Subject Group	N	Mean Levels of NOx	SD	*F*	*p*
Control	11	2095.91	281.98	5.72	0.008
P1	11	2259.00	119.99		
P2	11	2403.64	207.23		

P1= treatment with 15 mL syrup ; P2 = treatment with 30 mL syrup; SD = Standard deviation

production of nitric oxide which results to endothelial dysfunction. Nitric oxide can contribute to reperfusion tissues when produced in large amounts during reperfusion reacts with superosida to produce damaging oxidant peroxynitrite. [5,27]

In this study, the sample was moderate smokers that have a habit of smoking cigarettes as much as 10-19 cigarettes per day. Cigarette smoke contains a variety of reactive compounds that can lead to a state of oxidative stress in the body.[16] The use of the body's antioxidant reserves in an attempt to detoxify free radicals with a large number of smokers resulting deficiency in the body is quite heavy, thus predisposing to the development of a disease and the aging process.[21]

An imbalance between antioxidants and oxidants potentially leading to oxidative stress, has been associated with the pathogenesis of many diseases, including cancer, aging, atherosclerosis, ischemic injury, inflammation and degenerative diseases.[3,21] Free radicals have a very high reactivity and have a tendency to form a new radical when meeting with other molecules. This reaction will stop when there is an antioxidant compounds.[10]

Ipomoea batatas contains very high antioxidant compounds, namely anthocyanin. Anthocyanin is one of the antioxidants that can prevent various types of damages caused by oxidative stress, protects cells from free radicals[4]. Anthocyanin is a powerful antioxidant that can take a superoxide radical ($O2^{\bullet-}$), hydroxyl (OH•), lipid peroxyl ($ROO^{\bullet}$).[4].Antioxidant capacity of purple sweet potato can also help the antioxidant defense system in the body. Theoretically, the long-time smokers found that reduced levels of endogenous antioxidants. Intake of anthocyanin which is a non-enzymatic antioxidant flavonoid may prevent damage caused by free radicals by breaking the chain reaction of free radicals. As a result, free radicals oxidize antioxidants produce more stable and less reactive radical.[28] The antioxidants stabilize reactive oxygen compounds by reacting with formation of reactive radical. Anthocyanin is also inducing an antioxidant defense system of the body cells[17]. Use of an endogen antioxidant reserves in an attempt to detoxify free radicals in the body of a large number of smokers resulted in a severe enough deficiency, thus predisposing to the development of a disease and the aging process.[21]

Intake of anthocyanins as a non-enzymatic antioxidants can inhibit the reactivity of free radicals. The balance of antioxidants and oxidants are very important because it relates to immune function and cellular defenses. In addition to consuming free radicals directly, anthocyanins may help the antioxidant defense system by enhancing the function of endogenous antioxidants[17]. In conditions of stress, the anthocyanin can maintain the balance between antioxidants and oxidants, thus the oxidative damage can be prevented as well as the destruction of the body by free radicals.[15] Thus, the conditions can help maintain health, prevent diseases such as cancer, atherosclerosis as well as the process of aging and all diseases associated with aging.

This study supported by the previous study stating that the mice undergo oxidative stress treated with purple sweet potato extract showed a significant decrease in the levels of MDA.[11] Other study also proved that treatment with anthocyanin from purple eggplant skin extract could reduce the levels of MDA in the blood of mice with maximal activity[1]. This is due to anthocyanin in the purple sweet potato tubers act as antioxidants and free radical scavengers. It may play an important role in preventing the onset of aging, cancer and degenerative diseases such as arteriosclerosis.

Intake of anthocyanins as antioxidants can take free radicals such as superoxide and other reactive compounds. Anthocyanins are included in the class of flavonoids that are non-enzymatic antioxidants can inhibit the reactivity of free radicals. The balance of antioxidants and oxidants are very important because it relates to immune function and cellular defense. In addition to consuming free radicals directly, anthocyanin antioxidant defense system may help to improve the functioning of endogenous antioxidants [17]. In the absence of stress, there is a balance between antioxidants and oxidants that cause oxidative damage can be prevented as well as the destruction of the body by free radicals.[15] In other words, the condition can help to maintain health, prevent diseases such as cancer, atherosclerosis as well as the aging process and all the diseases associated with aging.

CONCLUSION

Consumption of the Ipomea batatas syrup, 15 mL or 30 mL twice a day for 2 weeks significantly reduced the MDA plasma levels and increase of NO_x plasma levels of moderate smokers in Denpasar-Bali. The consumption of the Ipomea batatas syrup appropriately can be considered to reduce the oxidative stress in moderate smokers.

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