GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Efficacy of *Ipomoea batatas L.* anthocyanin in reducing mammary *Malondialdehyde* levels in female rats exposed to tobacco smoke

Marini Atika Rahman [1], Astika Gita Ningrum [2,*], Baharika Suci Dwi Aningsih [3], Syahrida Wahyu Utami [4], Retty Ratnawati [5], I Wayan Arsana Wiyasa [6], Linda Purwaningsih [1] and Renata Alya Ulhaq [2]

[1] *Midwifery Study Program, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia.*
[2] *Midwifery Study Program, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.*
[3] *Midwifery Study Program, STIK Sint Carolus, Jakarta, Indonesia.*
[4] *Midwifery Study Program, STIKES Abdi Persada, Banjarmasin, Indonesia.*
[5] *Department of Physiology, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia.*
[6] *Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia.*

GSC Biological and Pharmaceutical Sciences, 2023, 23(02), 069–073

Publication history: Received on 31 March 2023; revised on 07 May 20213 accepted on 10 May 2023

Article DOI: https://doi.org/10.30574/gscbps.2023.23.2.0187

Abstract

Objectives: Tobacco smoke is one source of free radicals that can enter the body through the respiratory tract and circulate through the bloodstream to reach all organs in the body. Purple sweet potato (*Ipomoea batatas L.*) anthocyanin is a potentially powerful antioxidant that reduces free radicals. This study aims to demonstrate that purple sweet potato anthocyanin can reduce mammary gland malondialdehyde concentrations in female white rats (*Rattus norvegicus*) exposed to tobacco smoke.

Methods: This was experimental research with a randomized post-test control group. Thirty female white rats were used in this study. These samples were then divided into two control groups (negative control group - no exposure to tobacco smoke and anthocyanin; positive control group - exposure to tobacco smoke only -) and three exposure groups of exposure to cigarette smoke at a rate of two sticks/day and variety Anthocyanin doses for eight weeks. The malondialdehyde content of the mammary gland was measured spectrophotometrically.

Results: The result showed that the higher dose of *Ipomoea batatas* L anthocyanin administered, the lower the MDA level in the breasts of rats exposed to cigarette smoke (ANOVA test, p-value = 0.002)

Conclusions: *Ipomoea batatas L.* anthocyanin can decrease the concentration of mammary gland malondialdehyde in female rats exposed to tobacco smoke.

Keywords: Anthocyanin; Malondialdehyde; Tobacco use; Reproductive health

1. Introduction

Many countries consider smoking to be a severe hazard to human health, including Indonesia. According to the 2021 Global Adult Tobacco Survey, about 70,2 million Indonesian adults used tobacco (smoked, smokeless or heated tobacco products) [1]. According to the 2013 Indonesian Baseline Health Survey 2013, 85.0% of Indonesian households are exposed to tobacco smoke, and about eight smokers died from active smoking. Passive smokers died from secondhand smoke [2]. Tobacco smoke includes oxidizing chemicals that can promote free radical production. Free radicals are

* Corresponding author: Astika Gita Ningrum

reactive oxygen species in the body (ROS). ROS play a physiological role in the body under normal conditions. However, when ROS production increases, ROS and antioxidant imbalance can lead to oxidative stress [3, 4].

Exposure to cigarette smoke, one of the causes of oxidative stress, may be a risk factor for breast cancer. Chemical compounds in cigarette smoke can quickly enter the lungs when inhaled. Inhaled chemical compounds reach the blood vessels and then move with the blood circulation to the tissues in all parts of the body [5]. Free radicals from tobacco smoke, which are highly reactive, can react with phospholipids in cell membranes. The lipid peroxidation process produces a metabolite called malondialdehyde (MDA), which can be measured as an indicator of cell damage [6, 7]. Excess free radicals in the body are neutralized by free radicals known as antioxidants scavenger enzymes such as superoxide dismutase (SOD), catalase and/or glutathione peroxidase. However, if the proportion of free radicals is higher, oxidative stress cannot be avoided [6].

Anthocyanins are one bioactive substance that can be used as an antioxidant against free radicals. Anthocyanins donate electrons to free radicals and can also increase antioxidant activity (scavenger enzyme) in the body to neutralize free radicals [8–10]. Based on the foregoing, the purpose of this study is to illustrate the effect of purple sweet potato anthocyanins on MDA levels in a female rat's breast exposed to cigarette smoke.

2. Materials and methods

The research design used in this study was an experimental laboratory with a randomized, post-test-only method. The study was conducted from June to August 2016 at the Laboratory of Pharmacology and Laboratory of Physiology, Faculty of Medicine, Universitas Brawijaya, Malang,

The study's test animal was a female white rat, *Rattus norvegius* strain Wistar. The test animals were fed and hydrated as needed, the rats were housed in cages 20x30x40 cm. We utilized 30 rats that were 1.5-2 months old and weighed 150-200 grams. Within a week, test animals adjust to their surroundings. In addition, the test animals were divided randomly into five groups, each consisting of six rats. The control groups were as a positive control group exposed to tobacco smoke (two sticks/day) for eight weeks without anthocyanin and negative control group not exposed to tobacco smoke and without anthocyanin. Others groups (three groups) were exposed to tobacco smoke (two sticks/day) with doses anthocyanins of 20 mg/kgBW/day, 40 mg/kgBW/day, and 80 mg/kgBW/day orally by gavage for eight weeks.

Test animals were exposed to cigarette smoke at the Laboratory of Pharmacology, Faculty of Medicine, Universitas Brawijaya. Test animals were exposed to tobacco smoke for eight weeks when two cigarettes per day (each cigarette ± 4 minutes) were given in the morning (1 stick) and in the afternoon (1 stick) via smoke pump [11]. Gudang Garam Kretek Cigarettes, a well-known Indonesian cigarette brand popular among locals, were utilized.

Purple sweet potato (*Ipomoea batatas* L.) anthocyanins were used in this studyIt was produced using modified flash column chromatography with a stationary phase of polyamide CC-6 and mobile phases of water and ethanol. It was done at the Chemical Laboratory, Faculty of Mathematics and Science, Bandung Institute of Technology [12].

After eight weeks of treatment, rats were anesthetized with 1% ketamine. Intramuscular injection of 0.9 mL of distilled water and 0.1 mL of ketamine. Then waited until the rats ceased to move. In addition, The experimental animal was positioned on the table with its stomach up. Tapes affixed to the soles of the rats' four paws were used to secure them to the table.The next surgery is then performed to take the rat's breast tissue. The breast tissue is then stored in a container to examine MDA levels further. MDA in breast tissue was determined using a BIOXYTECH MDA-586TM malondialdehyde spectrophotometric test. Up to 200-300 mg of breast tissue was homogenized by chopping and grinding in a mortar and pestle, adding 1 ml of PBS, and then centrifuged at 6000 rpm for five minutes in an Evendorf. The prepared, coded test tube was then filled with 200 µL of sample or standard and 640 µL of reagent solution R1, followed by 10 L of probucol. It was vortexed until homogenous and incubated at 45 °C for 60 minutes before being centrifuged at 10,000 rpm for 10 minutes to get a clear supernatant, which was transferred to a cuvette. The resultant supernatant was then spectrophotometrically analyzed at 586 nm.

3. Results and Discussion

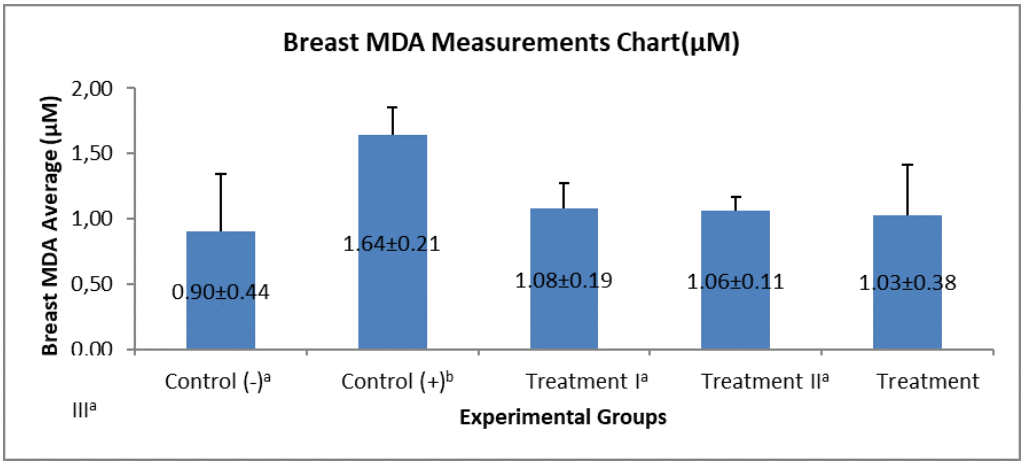

Figure 1 Breast MDA Levels of *Rattus norvegicus* Average in all experimental Group (µM)

3.1. Information

Diffelrelnt notation (a,b) indicates a significant meaning (p-value <0.05), and if the notation contains the same letter indicates there is no significant difference (p-value >0.05) *post hoc LSD test*. Control (-): rats without exposure to cigarette smoke and Anthocyanin. Control (+): rats exposed to 2 sticks/day of cigarette smoke but without Anthocyanin, Treatment I: administration of anthocyanin dosel of 20 mg/kgBW/day to rats exposed to two sticks/day of cigarette smoke, Trelatmelnt II: administration of anthocyanin dosel of 40 mg/kgBW/day to rats exposed to cigarette smoke two cigarettes/day, Trelatmelnt III: administration of anthocyanin dosel of 80 mg/kgBW/day to rats exposed to cigarette smoke two sticks/day.

Tobacco smoke inhaled by the body then becomes free radicals, which are very dangerous for the body because they can cause oxidative stress conditions, cause cell damage, and trigger a malignant tumor [6]. The human body produces antioxidants to combat the reactivity of free radicals. Because these antioxidants trap free radicals and prevent their reactivity from interrupting by breaking the chain reaction of free radicals with cellular components, they are also known as free radicals. The parts of cigarette smoke that enter the body and lungs then enter the blood vessels and travel with the bloodstream to the tissues of all body parts, including the breasts [5]. The accumulation of free radicals, which is relatively large, can lead to an increase in lipid peroxide, which also increases MDA levels. Therefore, a high concentration of MDA indicates a high level of free radicals in the body [11, 13]

The results showed that exposure to tobacco smoke could induce oxidative stress in the breasts of female rats. It was marked by a significant increase in the breast of female rats, which was a significant increase in the mammary MDA level compared to the negative control group not exposed to cigarette smoke (*p-value* <0,05). Cigarette smoke, an exogenous free radical that may enter the circulation and affect all body cells and tissues, can induce a rise in breast MDA. Cigarette smoke contains a high concentration of free radicals, which increases intracellular ROS concentration. The action and elevation of ROS cause oxidative stress, which destroys lipids, proteins, and DNA [14]. In DNA, malondialdehyde can bind to deoxyadenosine. It has the ability to generate mutant DNA in bodily cells. In this case, MDA may be regarded to have an effect on the carcinogenesis process [15].

MDA levels in the treatment group receiving purple sweet potato anthocyanins at three different doses were lower than the positive control group receiving no anthocyanins (p-value <0.05). The results of this study are consistent with those of other studies showing that anthocyanins can reduce oxidative stress conditions in the reproductive organs of male rats [12, 16]. This study also found that a 40 mg/kg body weight anthocyanin dosage might lower MDA levels in rats exposed to cigarette smoke.

This study shows that purple sweet potato anthocyanin can be a powerful antioxidant to prevent oxidative stress induced by cigarette smoke. Anthocyanins can act as antioxidants by reducing free radicals and scavenging free radicals by donating electrons to free radicals [9, 17, 18] Anthocyanin is a type of polyphenol among flavonoids that can directly or indirectly reduce oxidative damage by preventing the increase of free radicals in the body. Anthocyanin is a flavonoid and a polyphenol that acts as an antioxidant. As a strong antioxidant, anthocyanin can act as a reductant in the electron transfer reaction pathway by donating electrons to unpaired free radicals [19, 20]. Anthocyanins can also increase total

antioxidation capacity (T-AOC), which are enzymatic antioxidants (SOD and GSH-PX) in the body, and lower MDA levels so that oxidative stress would not occur in body organs [8, 21]. Further studies are needed to determine the lowest dose that can reduce malondialdehyde concentrations in *Rattus norvegicus* exposed to tobacco smoke. Further research is needed to determine whether purple sweet potato anthocyanins may also affect carcinogenic compounds found in tobacco smoke.

4. Conclusion

The administration of purple sweet potato anthocyanins (*Ipomoea batatas L*) in doses of 20, 40, and 80 mg/kg/day can reduce levels of malondialdehyde (MDA) breast *Rattus norvegicus* females exposed to cigarettes smoke.

Compliance with ethical standards

Disclosure of conflict of interest

Authors declare no conflict of interest.

Statement of ethical approval

This study has received ethical approval from the Faculty of Medicine, Universitas Brawijaya, Indonesia.

References

[1] World Health Organization (WHO). GATS|GLOBAL ADULT TOBACCO SURVEY Fact Sheet Indonesia 2021 GATS Objectives. Fact Sheet Indones 2021; 1–2.

[2] Ministry of Health Indonesia. Basic Health Research. Jakarta, Indonesia: Ministry of Health Indonesia, 2013.

[3] Wardani DWKK, Ali M, Khotimah H, et al. The effect of Centella asiatica to the vascular endothelial growth factor and vascular endothelial growth factor receptor-2 on the rotenone induced zebrafish larvae (Danio rerio) stunting model. GSC Biol Pharm Sci 2018; 5: 088–095.

[4] Halliwell B, C JM, Gutteridge. Free Radicals in Biology and Medicine. fifth edit. Oxford University Press, https://doi.org/10.1093/acprof:oso/9780198717478.001.0001 (2015, accessed 18 January 2023).

[5] Centers for Disease Control and Prevention (US), (US) NC for CDP and HP, (US) O on S and H. How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease: A Report of the Surgeon General. Atlanta (GA): Centers for Disease Control and Prevention (US). Centers for Disease Control and revention (US), https://www.ncbi.nlm.nih.gov/books/NBK53017/ (2010, accessed 18 January 2023).

[6] Sudiana IK. Patobiologi Molekuler Kanker. Jakarta: Salemba Medika, https://opac.perpusnas.go.id/DetailOpac.aspx?id=308734 (2011).

[7] Geum JL, Hwan WC, Ki HL, et al. Antioxidant Vitamins and Lipid Peroxidation in Patients with Cervical Intraepithelial Neoplasia. J Korean Med Sci 2005; 20: 267.

[8] Zhao J-G, Yan Q-Q, Lu L-Z, et al. In Vivo Antioxidant, Hypoglycemic, and Anti-tumor Activities of Anthocyanin extracts from Purple Sweet Potato. Nutr Res Pract 2013; 7: 359–365.

[9] Turner MK. Anthocyanins Increase Antioxidant Enzyme Activity in HT-29 Adenocarcinoma Cells. University of Goergia, https://www.google.com/search?q=6.+Turner%2C+Martha+Kathleen.+2009.+Anthocyanins+Increase+Antioxidant+Enzyme+Activity+in+HT-29+Adenocarcinoma+Cells.+Journal+of+Nutritional+Biochemistry%3A+1-4.&rlz=1C1GCEA_enID1030ID1030&oq=6.%09Turner%2C++Martha+Kathleen. (2007, accessed 18 January 2023).

[10] Utami SW, Ratnawati R, Wiyasa IWA. Effect of purple variety sweet potato (*Ipomoea batatas* L.) anthocyanin on expression of estrogen receptor-A and endometrium thickness on uterus of female white rats (*Rattus norvegicus*) exposed to cigarette smoke. Iran J Pharm Sci; 14, https://www.google.com/search?q=15.+Wahyu+Utami+S%2C+Ratnawati+R%2C+Wiyasa+IWA.+Effect+of+purple+variety+sweet+potato+(Ipomoea+batatas+L.)+anthocyanin+on+expression+of+estrogen+receptor-A+and+endometrium+thickness+on+uterus+of+female+white+rats+(Rattus+No (2018, accessed 18 January 2023).

[11] Winarsi H. Natural antioxidants and free radicals: Potential and applications in health, https://www.researchgate.net/publication/323104647_Antioxidants_natural_dan_radical_gratis_Potensi_dan_aplication_in_health (2007, accessed 18 January 2023).

[12] Jang H, Kim SJ, Yuk SM, et al. The Changes of Testis and the Effects of Anthocyanin on Spermatogenesis in Rat Induced Varicocele. Korean J Androl 2011; 29: 33.

[13] Ningrum AG, Frety EE, Diah I, et al. Antioxidant activity of purslane (Portulaca oleracea l.) leaf extract on the levels of ovarian oxidative stress and reproductive hormone in *Rattus norvegicus* exposed to cigarette smoke. Open Access Maced J Med Sci 2021; 9: 1535–1540.

[14] Ďuračková Z. Some current insights into oxidative stress. Physiol Res 2010; 59: 459–469.

[15] Mellor DD, Hamer H, Smyth S, et al. Antioxidant-rich spice added to hamburger meat during cooking results in reduced meat, plasma, and urine malondialdehyde concentrations. Am J Clin Nutr 2010; 92: 996–997.

[16] Jang H, Bae WJ, Kim SJ, et al. The Effect of Anthocyanin on the Prostate in an Andropause Animal Model: Rapid Prostatic Cell Death by Apoptosis Is Partially Prevented by Anthocyanin Supplementation. World J Mens Health 2013; 31: 239.

[17] Suda I, Oki T, Masuda M, et al. Physiological Functionality of Purple-Fleshed Sweet Potatoes Containing Anthocyanins and Their Utilization in Foods. Japan Agric Res Q JARQ 2003; 37: 167–173.

[18] Aningsih BSD, Sujuti H, Mustofa E, et al. Anthocyanins from *Ipomoea batatas* L. effect on ovarian malondialdehyde and 17β-estradiol in rats exposed cigarette smoke. Russ Open Med J; 9, https://romj.org/2020-0308 (2020, accessed 18 January 2023).

[19] Khoo HE, Azlan A, Tang ST, et al. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food Nutr Res; 61. Epub ahead of print 2017. DOI: 10.1080/16546628.2017.1361779.

[20] Wei J, Zhang G, Zhang X, et al. Anthocyanins Delay Ageing-Related Degenerative Changes in the Liver. Plant Foods Hum Nutr 2017; 72: 425–431.

[21] Linda Purwaningsih, Baharika Suci Dwi Aningsih, Syahrida Wahyu Utami, et al. The effect of anthocyanin of purple sweet potato (*Ipomoea batatas* L) on cervical malondialdehyde levels of female rats (*Rattus norvegicus*) exposed to cigarette smoke. GSC Biol Pharm Sci 2022; 19: 232–236.