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

The effect of anthocyanin of purple sweet potato (*Ipomoea Batatas* L) on cervical malondialdehyde levels of female rats (*Rattus Norvegicus*) exposed to cigarette smoke

Linda Purwaningsih [1], Baharika Suci Dwi Aningsih [2,*], Syahrida Wahyu Utami [3], Astika Gita Ningrum [4], Retty Ratnawati [5], Umi Kalsum [6] and Tatit Nurseta [7]

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

GSC Biological and Pharmaceutical Sciences, 2022, 19(03), 232–236

Publication history: Received on 14 May 2022; revised on 16 June 2022; accepted on 18 June 2022

Article DOI: https://doi.org/10.30574/gscbps.2022.19.3.0239

Abstract

Cigarette smoke contains oxidants that generate the production of free radicals. Free radical is a form of *Reactive Oxygen Species* (ROS). However, an increase in ROS production will cause an imbalance between ROS and antioxidants, leading to oxidative stress. Oxidative stress will induce lipid peroxidation, measurable by *malondialdehyde* (MDA) concentration. Anthocyanin is an exogenic antioxidant able to inhibit ROS. This study aimed to prove the effect of anthocyanin obtained from purple sweet potato to decrease MDA concentration in the cervix uterine of female rats (*Rattus norvegicus*) exposed to cigarette smoke. This study was an experimental study with a *Randomized Post Test Only Control Group Design on* 30 white female rats weighing 150-200 grams and aged 1.5-2 months, divided into 2 control groups and 3 treatment groups. One group was exposed to cigarette smoke (2 cigarettes/day) for 8 weeks without anthocyanine as the positive control group, and one group was not exposed to either cigarette smoke or anthocyanin as the negative control group. The 3 treatment groups received cigarette smoke exposure (2 cigarettes/day) and were given anthocyanin with a dosage of 20, 40, and 80 mg/kg/day, respectively, for 8 weeks. The concentration of cervical MDA was measured by the spectrophotometric method. The result showed a significant difference in MDA concentration between the positive control and the negative control groups with a $P<0.05$. There was no significant difference between the 20 and 40 mg/kg treated groups compared to the positive control group. The 80 mg/kg anthocyanin-treated group showed a significant difference in MDA cervix uterine concentration compared to the positive control group with a $P<0.05$.

Keywords: Cigarette Smoke; Anthocyanin; Purple Sweet Potato; Malondialdehyde; Cervix Uterine

1. Introduction

Smoking is a serious threat to human health, currently being faced by several countries, including Indonesia. The Global Adult Tobacco Survey in 2011 states that in Indonesia, about 225,000 people die each year due to smoking, and more than 97 billion become passive smokers [1]. Based on Basic Health Research of Indonesia (RISKESDAS) in 2013, 85% of households in Indonesia are exposed to cigarette smoke, with an estimated eight smokers dying from active smoking and one passive smoker dying from exposure to secondhand smoke[2].

* Corresponding author: Baharika Suci Dwi Aningsih
Bachelor of Midwifery Program, STIK Sint Carolus, Central Jakarta, Indonesia. Address of affilation: (STIK Sint Carolus, Salemba Raya Number 41, Central Jakarta, 10440, Indonesia.

Smoke from burning cigarettes contains oxidant compounds that can increase the production of free radicals. Free radicals are a form of Reactive Oxygen Species (ROS) in the body. Under normal circumstances, ROS in the body roles in physiological processes. However, if there is an increase in ROS production, it will cause an imbalance between ROS and antioxidants that can cause oxidative stress[3,4].

Oxidative stress results in lipid peroxidation, which can be determined by measuring malondialdehyde (MDA), the final product formed from the reaction of ROS with fatty acids from cell membranes. Lipid peroxidation will cause the chain of fatty acids to break into various toxic compounds and cause damage to cell membranes[5].

The endogenous antioxidant system may overcome oxidative stress by increasing the mechanism of action of enzymatic antioxidants. However, it takes an intake of antioxidants from outside the body to help protect the body and stabilize free radicals, which help overcome oxidative damage caused by free radicals in the body. One of the exogenous antioxidants that can be believed to have an excellent antioxidant effect because it can inhibit ROS, which is the cause of oxidative stress, one of which is anthocyanins [6].

One of the plants that contain anthocyanins is a purple sweet potato. Anthocyanin compounds found in purple sweet potato are known to function as antioxidants. Based on research conducted by Jang et al. (2011), the administration of anthocyanins can reduce the concentration of 8-hydroxy-2'-deoxyguanosine (8-OhdG), a marker of oxidative stress after male rats have varicocele [7,8].

Based on the description above, this study aimed to prove the effect of giving purple sweet potato anthocyanins to decrease cervical MDA levels in female rats (*Rattus norvegicus)* exposed to cigarette smoke.

2. Material and methods

It was an experimental research with the Post Test Only Control Group Design method. The research was conducted at the Pharmacology Laboratory and Physiology Laboratory, Faculty of Medicine, Universitas Brawijaya, Malang, in May-August 2016.

The experimental animal used in this study was a female white rat, *Rattus Norvegius* Strain Wistar. Experimental animals were given food and drink ad libitum and were kept in cages measuring 20×30×40 cm. The rats used were 30 with criteria of 1.5-2 months of age and 150-200 grams of body weight. Experimental animals were acclimatized for 1 week to adapt to their environment. Furthermore, the experimental animals were randomly divided into 5 groups, with 6 rats in each group. The control groups were 1 group exposed to cigarette smoke (2 cigarettes/day) for 8 weeks and without anthocyanins as a positive control group, and 1 group not exposed to cigarette smoke and without anthocyanin as a negative control group. The 3 treatment groups were given exposure to cigarette smoke (2 cigarettes/day) with anthocyanins (doses of 20, 40, and 80 mg/kg BW/day) for 8 weeks, orally using a probe.

Exposure to cigarette smoke in experimental animals was conducted at the Pharmacology Laboratory, Faculty of Medicine, Universitas Brawijaya. Exposure to cigarette smoke in experimental animals was carried out for 8 weeks, given 2 cigarettes/day (each cigarette ± 4 minutes) in the morning (1 stick) and afternoon (1 stick) using a smoking pump [9]. The cigarettes used were famous Indonesian cigarette brands commonly smoked by locals', i.e., Gudang Garam Kretek Cigarettes.

This study used anthocyanins from purified purple sweet potato (*Ipomoea batatas* L.). It was obtained through modified flash column chromatography with polyamide CC-6 stationary phase, using water and ethanol as mobile phases, conducted in the Chemistry Laboratory, FMIPA ITB [10]. According to the dose of anthocyanin in each group, they included Group A1: 20 mg/kg BW, Group A2: 40 mg/kg BW, and Group A3: 80mg/kg BW. Anthocyanin solution was taken using a 1 ml syringe and then force-fed into the rat's stomach using a probe.

After 8 weeks of treatment, the rats were terminated by anesthesia by giving 1% ketamine with a dose of 0.9 ml of distilled water plus 0.1 ml of ketamine, injected intramuscularly, then waited until the rats did not move anymore. Furthermore, the experimental animal was placed on the board with the stomach facing up. The rats were placed on the board using tacks that were plugged into the four soles of their feet. Next, the rat's abdominal wall was carefully opened using tweezers and scissors with an incision in the midline, followed by the left and right sides at the top and bottom, and the diaphragm was opened. Then, the cervix was taken.

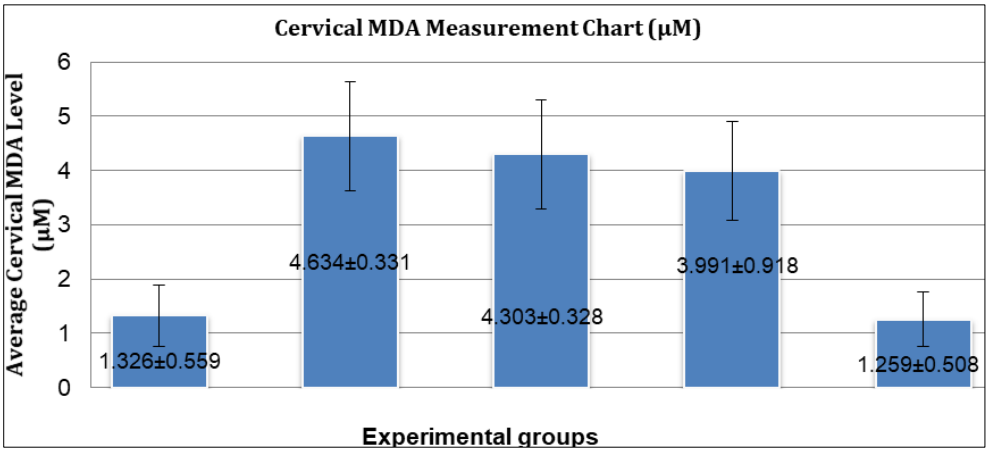
Cervical MDA was measured using BIOXYTECH MDA-586TM Spectrophotometric Assay for Malondialdehyde. 200-300 mg Cervical tissue was homogenized by chopping and grinding using a mortar and adding 1 ml of PBS, then put into

Evendorf, vortexed, centrifuged at 6000 rpm for 5 minutes, and then prepared a tube that has been coded and given 10 l probucol, added 200 µl sample or standard and 640 reagent solution R1. It was vortexed to make it homogeneous and incubated at 45 ℃ for 60 minutes, then centrifuged at 10,000 rpm for 10 minutes to produce a clear supernatant, transferred the supernatant to a cuvette. The supernatant obtained was then measured using spectrometry with a wavelength of 586 nm.

3. Results and discussion

The results of cervical MDA measurements obtained in Figure 1 show that the effect of exposure to cigarette smoke on cervical MDA levels in the two control groups (the negative control group and the positive control group) had a significant difference between the two with a significance value of p= 0.000 (p <0.05).

The intervention of purple sweet potato anthocyanins (*Ipomoea batatas L.*) could reduce cervical MDA levels in rats exposed to cigarette smoke. Figure 1 shows that cervical MDA levels between the positive control group and the A1 and A2 doses had decreased cervical MDA, but there was no significant difference (p>0.05). However, if the positive control group was compared to the A3 dose group, there was a significant difference between the two with a significance value of p=0.000 (p<0.05).



Notes: Different notations (a, b) show a significant meaning (p <0.05), the same notations indicate no significant difference (p >0.05) based on the post-hoc-tukey test. Negative Control: Without exposure to cigarette smoke and anthocyanins, Positive Control: Exposure to 2 cigarettes smoke/day for 8 weeks and without anthocyanins, Dose I (20 mg/kg BW/day) exposure to cigarette smoke 2 sticks/day and anthocyanin at a dose of 20 mg/kg BW/day for 8 weeks, Dose II (40 mg/kg BW/day) exposure to cigarette smoke 2 cigarettes/day and anthocyanin at a dose of 40 mg/kg BW/day for 8 weeks, Dosage III (80 mg/kg BW/day) exposure to cigarette smoke 2 sticks/day and anthocyanin at a dose of 80 mg/kg BW/day for 8 weeks

Figure 1 Average Cervical MDA Levels of *Rattus norvegicus* in all experimental groups

Cigarette smoke is a form of free radicals that contains several free radical components consisting of toxic gases, carcinogenic components, volatiles, semivolatile components, and several forms of ROS, including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-), which can cause oxidative damage [11].

This study found that exposure to cigarette smoke in female rats could cause a significant increase in cervical MDA levels compared between the positive control group and the negative control group (p <0.05). The increase in cervical MDA can be caused by exposure to cigarette smoke, a form of exogenous free radicals, which contains ROS and carcinogenic components that can cause oxidative stress.

ROS produced by cigarette smoke will work to increase the destruction of endogenous antioxidants (vitamins and enzymatic antioxidants) so that they can reduce the critical role of cellular antioxidants in building a defense. ROS will react with phospholipids that make up the cell membrane system so that malondialdehyde (MDA) can be formed [11,12].

In the body's cells, MDA can react with deoxyadenosine and deoxyguanosine in DNA and form mutagenic DNA. In the presence of this condition, it can be stated that MDA can have implications for the process of casinogenesis [13].

Administration of exogenous antioxidants in the form of purple sweet potato anthocyanins with several different doses can reduce cervical MDA levels, which increase due to exposure to cigarette smoke. The dose group A3 (80 mg/kg BW) showed a significant decrease in cervical MDA levels compared to the positive control group.

The administration of purple sweet potato anthocyanin at a dose of 80 mg/kg BW/day was more effective than doses 1 and 2 in reducing cervical MDA levels. Based on the results of the decrease in cervical MDA, it was found that only giving a dose of 80 mg/kg BW/day could significantly reduce cervical MDA levels, although the administration of anthocyanins at doses of 20 mg/kg BW/day and 40 mg/kg BW/day also affected decreasing cervical MDA levels, but not significant. From the results of this study, it was found that the efficient and quick in lowering cervical MDA levels in female rats exposed to cigarette smoke was at a dose of 80 mg/kg BW compared to doses of 20 mg/kg BW/day and 40 mg/kg BW of anthocyanin.

This study is in line with previous research conducted by Jang et al. (2011), which proved that the administration of anthocyanins at a dose of 80 mg/kg BW could significantly reduce the concentration of 8-hydroxy-2'-deoxyguanosine (8-OhdG), a marker of oxidative stress in male rats with varicocele.[6] Likewise, research conducted by Zhao et al. (2013) states that the work of anthocyanins as antioxidants can increase enzymatic antioxidants in the body, such as GSH-PX and SOD. In addition, anthocyanins can reduce MDA levels, which is an indicator of oxidative stress [14,15].

4. Conclusion

Exposure to cigarette smoke 2 cigarettes/day for 8 weeks can increase cervical MDA levels in female white rats. The administration of purple sweet potato anthocyanins effectively reduces cervical tissue MDA levels in female white rats exposed to cigarette smoke. The administration of anthocyanins at a dose of 80 mg/kg BW to female white rats exposed to cigarette smoke significantly affected cervical MDA levels.

Further research is needed by expanding the measurements limited to free radicals and measuring chemicals or carcinogens produced from cigarette smoke.

Compliance with ethical standards

Acknowledgments

We present our gratitude to Dr. Retty Ratnawati, MSc; Dr. Umi Kalsum, M.Kes and Dr. Tatit Nurseta, SpOG (K) for the great thought, knowledge, guidance, support, advice and motivation during this research and journal writing.

Disclosure of conflict of interest

We warrant that the article is the Authors' original work and ensure no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is not under review at any other publication.

Statement of ethical approval

All procedures performed in studies involving animals were in accordance with the ethical standards at the University of Brawijaya, Malang, numbered 274/ EC/KEPK/07/2016.

References

[1] World Health Organization. Global Adult Tobacco Survey (GATS) [Internet]. Atlanta; 2020. Available from: https://cdn.who.int/media/docs/default-source/ncds/ncd-surveillance/gats/18_gats_analysispackage_final_23nov2020.pdf?sfvrsn=67e2065f_3

[2] Kementerian Kesehatan Republik Indonesia. Riset Kesehatan Dasar. Badan Penelitian dan Pengembangan Kesehatan. Jakarta; 2013.

[3] Halliwell B, Gutteridge JMC. Free Radicals in Biology Medicine. 5th ed. United Kingdom: Oxford Univercity Press; 2015.

[4] Wardani DWKK, Ali M, Khotimah H, Nurdiana, Riawan W, Primihastuti D, 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 Nov 30;5(2):088–95.

[5] Asni E. Pengaruh Hipoksia Berkelanjutan terhadap Kadar Malondialdehid, Glutation Tereduksi dan Aktivitas Katalase Ginjal Tikus. Universitas Indonesia; 2008.

[6] Wulansari D, Chairul. Penapisan Aktivitas Antioksidan Dan Beberapa Tumbuhan Obat Indonesia Menggunakan Radikal 2,2-Diphenyl-1 Picrylhydrazyl (Dpph) Antioxidant Screening Activity of Several Indonesian Medicinal Plants Using 2,2-Difenil 1-1 Picrylhidrazyl(Dpph). Maj obat Tradis. 2011;16(1):22–5.

[7] Jang H, Kim SJ, Yuk SM, Han DS, Ha US, Hong SH, et al. The Changes of Testis and the Effects of Anthocyanin on Spermatogenesis in Rat Induced Varicocele. 2011;29(1):33–42.

[8] Wahyu Utami S, 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. 2018;14(2):51–64.

[9] Rahmawati R, Saputri I, Ratnawati R, Soeharto S, Wiyasa I. The effect of α-tochoperol on Oxidative Stress and Ovarian Fuction in Rats Exposed to Tobacco Smoke. Cukurova Med J. 2014;39(2):203–112.

[10] S J, Ciptati. Ekstraksi, Isolasi, Pemurnian dan Karakterisasi Antosianin dari Ubi Jalar Ungu (Ipomoea batatas L). Pros Simp Nas Inov Pembelajaran dan Sains. 2013;367-370.

[11] Valavanidis A, Vlachogianni T, Fiotakis K. Tobacco smoke: Involvement of reactive oxygen species and stable free radicals in mechanisms of oxidative damage, carcinogenesis and synergistic effects with other respirable particles. Int J Environ Res Public Health. 2009;6(2):445–62.

[12] Winarsi H. Antioksidan Alami & Radikal Bebas. Kanisius; 2007.

[13] Mellor DD, Hamer H, Smyth S, Atkin SL, Courts FL. Antioxidant-rich spice added to hamburger meat during cooking results in reduced meat, plasma, and urine malondialdehyde concentrations. Am J Clin Nutr. 2010;92(4):996–7.

[14] Zhao JG, Yan QQ, Lu LZ, Zhang YQ. In vivo antioxidant, hypoglycemic, and anti-tumor activities of anthocyanin extracts from purple sweet potato. Nutr Res Pract. 2013;7(5):359–65.

[15] Aningsih BSD, Sujuti H, Mustofa E, Ratnawati R. Anthocyanins from Ipomoea batatas L. Effect on ovarian malondialdehyde and 17β-estradiol in rats exposed cigarette smoke. Russ Open Med J. 2020;9(3):1–5.