KEMENTERIAN PENDIDIKAN DAN KEBUDAYAAN
UNIVERSITAS AIRLANGGA
FAKULTAS KEDOKTERAN
PROGRAM STUDI ILMU KEDOKTERAN JENJANG DOKTOR
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Nomor : 342/UN3.1.1/DL/2020
Lamp :
Hal : Penyanggah Ujian Akhir Tahap 2 (Terbuka)

14 Januari 2020
B. Yanti
Arsip Terbuka

Kepada Yth.
Pimpinan Sidang Ujian Akhir Tahap 2 (Terbuka)
Program Studi Ilmu Kedokteran Jenjang Doktor FK UNAIR
Surabaya

Sehubungan dengan Ujian Akhir Tahap 2 (Terbuka) sdr. **Dina Helianti, dr., M.Kes** pada tanggal **20 Januari 2020**, maka dengan ini kami sampaikan nama-nama penyanggah ujian akhir yang bersangkutan untuk diketahui.

Pimpinan sidang ujian akhir terbuka: Prof. Dr. H. Budi Santoso, dr., Sp.OG(K)

Para penyanggah dimaksud adalah :

1. Prof. Soetjipto, dr., MS., Ph.D *)
2. Prof. Dr. Widjiati, drh., M.Si **) ✓ 1
3. Prof. Dr. A. Retno Pudji Rahayu, drg., M.Kes ✓ 2
4. Dr. Soedarsono, dr., Sp.P(K)
5. Supangat, dr., M.Kes., Ph.D., Sp.BA ✓ 3
6. Dr. Tjuk Imam Restiadi, drh., M.Si ✓ 4
7. Dr. Johanes Nugroho Eko Putranto, dr., Sp.JP(K) ✓ 5
8. Dr. Lestari Sudaryanti, dr., M.Kes ✓ 6
9. Prof. Dr. Siswandono, MS., Apt ✓ 7
10. Prof. Dr. H. Budi Santoso, dr., Sp.OG(K)

7 orang luar FK

Demikian dan atas perhatiannya disampaikan terima kasih.

a.n. Dekan
Wakil Dekan I,

Prof.Dr. David S. Perdanakusuma,dr.,Sp.BP-RE(K)
NIP. 196003051989011002

Catatan :
*) Promotor

sdh Prog

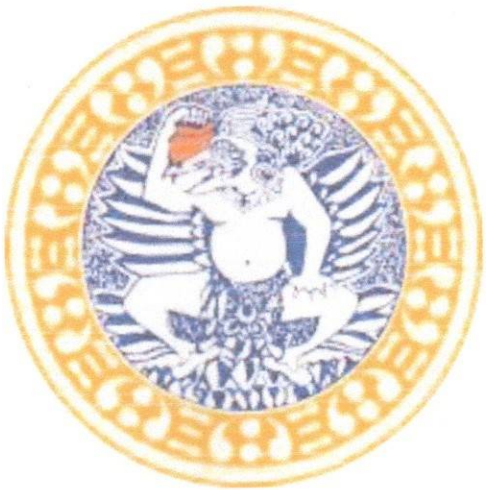
DAFTAR HADIR PENYANGGAH

Rapat/Sidang : Ujian Doktor Terbuka *Dina Helianti, dr., M.Kes*
Tanggal : 20 Januari 2020
Pukul : 10.00 – 12.00 Wib
Tempat : Aula Fakultas Kedokteran UNAIR
Acara : Penentuan Predikat Calon Doktor *Dina Helianti, dr., M.Kes*

No	Nama	Instansi	Tanda Tangan	
1.	Prof. Soetjipto, dr., MS., Ph.D		1.	
2.	Prof. Dr. Widjiati, drh., M.Si			2.
3.	Prof. Dr. A. Retno Pudji Rahayu, drg., M.Kes		3.	
4.	Dr. Soedarsono, dr., Sp.P(K)			4.
5.	Supangat, dr., M.Kes., Ph.D., Sp.BA		5.	
6.	Dr. Tjuk Imam Restiadi, drh., M.Si			6.
7.	Dr. Johanes Nugroho Eko Putranto, dr., Sp.JP(K)		7.	
8.	Dr. Lestari Sudaryanti, dr., M.Kes			8.
9.	Prof. Dr. Siswandono, MS., Apt		9.	

DISERTASI

MEKANISME PENCEGAHAN DISFUNGSI ENDOTEL OLEH KAKAO (*Theobroma cacao*) MELALUI ANALISIS F2-ISOPROSTAN PLASMA, EKSPRESI NFκB, CD-34, DAN Flk-1 PADA TIKUS PUTIH *STRAIN Sprague dawley* YANG TERPAPAR ASAP ROKOK



DINA HELIANTI

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FAKULTAS KEDOKTERAN UNIVERSITAS AIRLANGGA
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DINA HEIANTI

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DISERTASI

Untuk memperoleh Gelar Doktor
dalam Program Studi Ilmu Kedokteran Jenjang Doktor
pada Fakultas Kedokteran Universitas Airlangga
telah dipertahankan di hadapan
Panitia Ujian Doktor Terbuka

Pada hari : Selasa
Tanggal : 20 Januari 2020
Pukul : 10.00-12.00 WIB

Oleh :

DINA HELIANTI
011317017311

PROGRAM STUDI ILMU KEDOKTERAN JENJANG DOKTOR
FAKULTAS KEDOKTERAN UNIVERSITAS AIRLANGGA
SURABAYA
2020

LEMBAR PENGESAHAN

MEKANISME PENCEGAHAN DISFUNGSI ENDOTEL OLEH KAKAO (*Theobroma cacao*) MELALUI ANALISIS F2-ISOPROSTAN PLASMA, EKSPRESI NFκB, CD-34, DAN Flk-1 PADA TIKUS PUTIH *STRAIN Sprague dawley* YANG TERPAPAR ASAP ROKOK

TELAH DISETUJUI

PADA TANGGAL 20 JANUARI 2020

Oleh:
Promotor

Prof. Soetjipto, dr., MS., PhD
NIP. 195002171978031002

Kopromotor

Prof. Dr. Widjiati, drh., M.Si
NIP. 1962091519900220001

SUMMARY

THE PREVENTION MECHANISMS OF ENDOTHELIAL DYSFUNCTION BY CACAO (*Theobroma cacao*) THROUGH ANALYSIS OF PLASMA F2-ISOPROSTAN LEVELS, EXPRESSION OF NF-KB, CD-34, AND FLK-1 ON CIGARETTE SMOKING EXPOSED RAT

Cardiovascular disease (CVD) is the main cause of illness and death in the world. In 2008, deaths due to PKV in the world reached 30% of the total deaths at that time, and by 2030 it is estimated to increase to 37%. Smoking habits increase the risk of cardiovascular disease 2-3 times, while the risk of coronary heart disease 2-4 times. Indonesia is the third country with the highest number of smokers in the world (27.6%).

Cigarette smoke contains nicotine, CO, tar, as well as many types and amounts of oxidants that can help with various pathological effects, especially on the endothelium. The increase in ROS due to cigarette smoke causes lipid peroxidation in the endothelial cell membrane with the final product F2-isoprostane which can be used as an early indicator of the atherogenesis process. Cigarette smoke also causes an inflammatory process through the activation of Nuclear Factor kappa B (NFκB) which triggers an increase in pro-inflammatory cytokines and further causes activated endothelium which affects the expression of adhesion molecules such as ICAM-1, and VCAM-1 so that the anti-adhesive properties of the endothelium decrease which is a sign of endothelial dysfunction. Besides causing endothelial damage, cigarette smoke also interferes with the regeneration and maintenance of endothelium. Endothelial progenitor cells (EPCs) play a role in the process of endothelial regeneration either through the paracrine system (including Vascular Endothelial Growth Factor, Fibroblast Growth Factor, IL-6, IL-8, IL-11) or differentiate into endothelial cells. EPC markers that are often used are Cluster of differentiation 133+ (CD133 +), CD34 + and Vascular Endothelial Growth Factor Receptor 2+ (VEGFR2 +)/Fetal Liver Kinase-1 (Flk-1).

Epidemiological data suggest that regular intake of certain herbal plants can reduce the risk of CVD. Cocoa (Theobroma cacao) or chocolate is a food ingredient that has been shown to be beneficial for cardiovascular health. A study by Bayard et al., 2007 of residents of Kuna India off the coast of Panama who used to consume cocoa every day showed lower mortality from cardiovascular disease compared to citizens of other countries (9.2 ± 3.1 versus 83.4 ± 0.7 age-adjusted deaths per 100′000).

In vivo studies on the mechanism of cocoa administration on the prevention of endothelial dysfunction due to smoking, in this case, the increase in ICAM-1 and VCAM-1 has not been widely carried out, while research data on the effect of cocoa in smokers on EPC levels in the blood circulation have been reported. This study intends to investigate further the mechanisms for preventing endothelial dysfunction by cocoa due to cigarette smoke, particularly the relationship between oxidative stress, inflammation, and EPC activation with the recency of the immunohistochemical increase in EPC in the injured area, in this case, arteria coronaria. The oxidative stress pathway used an indicator of plasma F2-isoprostane levels, the inflammatory pathway by the expression of the NFkB coronary artery, and the EPC activation pathway by the expression of CD34 and Flk-1 coronary artery, while the endothelial dysfunction used the indicators ICAM-1 and VCAM-1.

This research was conducted in 2 phase. Phase 1 to determine the effective dose of cacao in reducing plasma F2-isoprostane levels; and phase 2 to analyze the mechanisms of preventing endothelial dysfunction by cacao on cigarette smoke exposure. In the first phase

of study, 3 doses of cacao were used. This type of phase 1 is an experimental laboratory with a post-test-only control group design model. The experimental animals used were 30 rattus norvegicus strains of Sprague Dawley, males, aged 3 months, weight 200-300 g grouped randomly into 5 treatment groups. The normal control group (K0) received 2 ml of aqua bidest and given air exposure once a day; the cigarette control group (K1) received 2 ml of aqua bidest and given exposure to cigarette smoke once a day. In the treatment group, each group was given exposure to cigarette smoke and given cocoa powder that had been dissolve in aqua bidest. Cacao group 1 was given a dose of 1206 mg/kg BW/day (P1), cacao group 2 was given a dose of 2411 mg/kg BW/day (P2), and cacao group 3 was given a dose of 3616 mg/kg BW/day (P3). Each group was treated for 14 days. On the 15th day, the experimental animals were terminated and F2-isoprostane was examined using the ELISA method, then was selected one of the three doses of cacao was the most effective in reducing plasma F2-isoprostane levels.

The result of phase 1 showed that the effective dose of cacao in reducing plasma F2-isoprostane levels was 1205 mg/kg BW/day. Furthermore, the cacao treatment group with a dose of 1205 mg/kg BW/day in the second phase of the study was called the treatment group (P), together with the K0 and K1 groups, the NFκB, CD34, Flk-1, VCAM-1 and ICAM-1 were examined. The results of examinations in phases 1 and 2 showed that cacao decreased the expression of NFκB, VCAM-1, and ICAM-1 arteria coronaria and increased the expression of CD34 and Flk-1 coronary artery in rats exposed to cigarette smoke. The result of the path analysis test showed that there was a relationship between cacao in the condition of exposure to cigarette smoke with an increase in CD34 and a decrease in ICAM-1. In addition, there is also a relationship between cacao and a decrease in NFκB and a decrease in VCAM-1.

New in this study, presenting cacao to Rattus norvegicus exposed to cigarette smoke can prevent endothelial dysfunction in 2 ways: the pathway of increasing EPC and an inflammatory pathway with a decrease in NFκB as an inflammatory mediator. The results of this study can be used as a basis for knowledge of prevention disease due to cigarette smoke by using cacao which can be applied daily.

ABSTRACT

The Prevention Mechanisms of Endothelial Dysfunction by Cacao (Theobroma Cacao) Through Analysis of Plasma F2-Isoprostan Levels, Expression of NF-kB, CD-34, and Flk-1 on Cigarette Smoking Exposed Rat

Dina Helianti

Background: Smoking has known as causative factor of cardiovascular disease that was started with endothelial dysfunction. Polyphenols has known significantly prevent endothelial dysfunction. Cacao is a rich source of polyphenols. This study was designed to evaluate the cardioprotective effects of cocoa that mediated through the anti-oxidant effect, and was measured by plasma F2-isoprostane level, anti-inflammatory effect by expression of NFκB, and Endothelial Progenitor Cell (EPC) activation by expression of CD-34 and Flk-1 in coronary arteries. The condition of endothelial dysfunction was measured by expression of ICAM-1 and VCAM-1 in coronary arteries.

Material and Methods: These research was conducted in 2 phases: the first phase determined the efective dose of cocoa in reducing plasma F2-isoprostane level and the second phase analyzed the preventing mechanism of endothelial dysfunction by cocoa on cigarette smoke exposure. This study using cocoa powder. In the first phase, 3 doses of cocoa were used. This study subjected rats, divided into five groups: the normal control group (2 ml of aquabidest, air exposure); the cigarette control group (2 ml of aquabidest, cigarette smoke); cacao group 1 (1205 mg/kg BW/day, cigarette smoke); cocoa group 2 (2410 mg/kg BW/day, cigarette smoke); cacao group 3 (3615 mg/kg BW/day, cigarette smoke). Each group was treated for 14 days. In the second phase of the study using the optimal dose of cacao, based on the results from the first phase. NFkB, CD34, Flk-1, VCAM-1 and ICAM-1 were measured by immunohistochemistry.

Results: Cocoa 1205 mg/kg/day significantly decreases plasma F2-isoprostane level, NFkB, ICAM-1 and VCAM-1 expression of coronary arteries in cigarette smoking exposed rat ($p<0,05$). There was not a significant increases CD-34 but there was a significant increases in Flk-1 expression ($p<0,05$).

Conclusions: Cocoa in cigarette smoke-exposed rats can prevent endothelial dysfunction in 2 ways: the pathway of increasing EPC and an inflammatory pathway with a decrease in NFκB as an inflammatory mediator. The results of this study can be used as a basis for preventing endothelial dysfunction due to cigarette smoke by using cocoa.

Keywords: cigarette smoke exposure, cacao, F2-isoprostane, EPC, endothelial dsyfunction