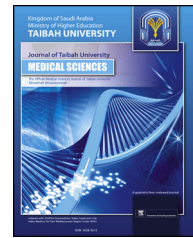




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Original Article

Effects of *Theobroma cacao* on heat shock protein 90 and asymmetric dimethylarginine of endothelial cells under the influence of plasma of pre-eclamptic patients

Ari Kusmiwiyati, MS^{a,*}, Siti C.W. Baktiyani, MD, PhD^b and Nurdiana Nurdiana, MD, PhD^c

^a Midwifery Diploma Study Programme, Health Polytechnic of Health Ministry, Malang, Indonesia

^b Obstetric and Gynecology Laboratory, Saiful Anwar General Hospital, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia

^c Pharmacology Laboratory, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia

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المخلص

أهداف البحث: أجريت هذه الدراسة للتحقق من الدور التنظيمي لمستخلص الإيثانول من فول ثيوبروما كاكاو على بروتين الصدمة الحرارية 90 وديمثيل أرجنين غير المتماثل للخلايا البطانية الناتجة من بلازما مريضات تسمم-الحمل.

طرق البحث: تم الحصول على الخلايا البطانية من السرة البشرية في المرحلة المتموجة، وتم تقسيم الخلايا إلى مجموعات؛ مجموعة التحكم (من غير علاج)، مجموعة الخلايا البطانية التي تعرضت إلى 2% من بلازما مريضات تسمم-الحمل، مجموعة الخلايا البطانية التي تعرضت إلى 2% من بلازما مريضات تسمم-الحمل وعولجت بمستخلص الإيثانول من ثيوبروما كاكاو بجرعات مختلفة (25؛ 50؛ 100). كما تم تحليل مستوى بروتين الصدمة الحرارية 90 بواسطة فحص الإنزيمات المناعية الماصة. تم تحليل التعرض لديمثيل أرجنين غير المتماثل باستخدام الكيمياء الخلوية المناعية.

النتائج: كان هناك انخفاض لمستوى بروتين الصدمة الحرارية 90 في المجموعة التي تعرضت للبلازما المأخوذة من مريضات تسمم-الحمل. وقد تم تثبيط هذا الانخفاض بشكل ملحوظ بمستخلص ثيوبروما كاكاو في الجرعات 50 و 100. عملت البلازما المأخوذة من مريضات تسمم-الحمل على زيادة كبيرة في ديمثيل أرجنين غير المتماثل بالمقارنة مع مجموعة التحكم. تم تثبيط هذه الزيادة بشكل ملحوظ بإعطاء مستخلص ثيوبروما كاكاو الذي تم إعطاؤه بجرعتين أعلى.

* Corresponding address: Midwifery Diploma Study Programme, Health Polytechnic of Health Ministry, Jl. Besar Ijen, No. 77, Malang, East Java, Indonesia.

E-mail: arikusmiwiyati@yahoo.com (A. Kusmiwiyati)

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الاستنتاجات: عمل مستخلص فول ثيوبروما كاكاو على حماية الخلايا البطانية، التي تعرضت لبلازما مريضات تسمم-الحمل بزيادة بروتين الصدمة الحرارية 90 وتقليل استخراج ديمثيل أرجنين غير المتماثل.

الكلمات المفتاحية: ديمثيل أرجنين غير المتماثل؛ مستخلص الإيثانول؛ بروتين الصدمة الحرارية 90؛ في المختبر؛ بروتين الإجهاد

Abstract

Objectives: This study was conducted to investigate the modulatory role of an ethanol extract of *Theobroma cacao* beans on heat shock protein 90 (HSP90) and asymmetric dimethylarginine (ADMA) levels of endothelial cells under the influence of plasma of pre-eclamptic patients.

Methods: The endothelial cells were obtained from a human umbilicus. In the confluent phase, the cells were subdivided into groups: the control group (no treatment), the endothelial cell group that was exposed to 2% pre-eclamptic patients' plasma, and the endothelial cell group that was exposed to 2% pre-eclamptic patients' plasma and treated with the ethanol extract of *T. cacao* at various doses (25, 50, or 100 ppm). Analysis of HSP90 levels was carried out by an enzyme-linked immunosorbent assay. Quantification of ADMA was conducted by immunocytochemistry.

Results: There was a decreased HSP90 level in the group exposed to the pre-eclamptic patients' plasma. This decrease was significantly attenuated by the extract of *T. cacao* at the doses of 50 and 100 ppm. The pre-

1 eclamptic patients' plasma significantly increased ADMA
2 expression as compared with the control group. This in-
3 crease was significantly attenuated by the administration
4 of the *T. cacao* extract at the two highest doses.

6 **Conclusions:** The extract of *T. cacao* beans protected the
7 endothelial cells that were exposed to pre-eclamptic pa-
8 tients' plasma by increasing HSP90 levels and reducing
9 ADMA levels.

11 **Keywords:** ADMA; Ethanol extract; HSP90; *In vitro*; Stress
12 protein

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22 Introduction

23 Pre-eclampsia is a syndrome that develops in pregnant
24 women in the form of hypertension and proteinuria after 20
25 weeks of pregnancy. This syndrome affects 3–5% of preg-
26 nancies and occurs due to a placental abnormality. Because it
27 affects only pregnant women, this disorder can lead to
28 complications, which may happen before or after childbirth
29 to the mother or the child. Pre-eclampsia is dangerous, and
30 early detection efforts and the best treatment of this disorder
31 are still not satisfactory.^{1,2} The final effect of this disorder is
32 expressed as the incomplete invasion degree of the
33 trophoblast and endothelial cell dysfunction, which trigger
34 placental dysregulation.³

35 Heat shock proteins (HSPs) are expressed by all cells.
36 These proteins have diverse functions; one of their functions
37 is to assist the cells in the defence against stress. Some HSPs are
38 constitutively expressed, and others are adaptively expressed
39 in response to specific injuries.⁴ Heat shock protein 90
40 (HSP90) is the most abundant chaperone, estimated at
41 approximately 1–2% of total cellular protein.⁵ Research has
42 proved that the increase in Hsp90 levels occurs in red blood
43 cells of pre-eclamptic patients' umbilical cord compared to
44 normotensive pregnant women.⁶ In pre-eclampsia, a decrease
45 in endothelial cell viability is associated with an increase in
46 HSP90 expression.⁷ Other studies have revealed that HSP90 is
47 down-regulated in peripheral blood of pre-eclamptic patients
48 with or without foetal growth restriction.⁸

49 Nitric oxide (NO) is also involved in the development of
50 pre-eclampsia. Anomalous regulation of NO metabolism is
51 caused by down-regulation of NO synthase owing to
52 competitive inhibition by asymmetric dimethylarginine
53 (ADMA).⁹ The latter is an endogenous competitive inhibitor
54 of NO synthase and plays a role in endothelial dysfunction.¹⁰
55 Various studies have confirmed that ADMA levels are
56 increased in pre-eclamptic patients^{11–17} although the
57 results of some studies are inconsistent.^{18,19} In fact, the
58 ADMA level is an indicator of the risk of pre-eclampsia in
59 pregnancy.²⁰ Considering that endothelial NO synthase
60 (eNOS) interacts with HSP90 as a regulatory protein,²¹
61 changes in NO regulation should alter HSP90 expression.

62 Until now, no studies have proved HSP90 regulation or its
63 relation to ADMA in endothelial cells of pre-eclamptic pa-
64 tients. Moreover, no studies have shown an influence of an
65 herbal ingredient on HSP90 and ADMA in endothelial cells
66 of pre-eclamptic patients.

67 *Theobroma cacao* is a plant currently used in beverages
68 and foods by people all over the world. *T. cacao* contains a
69 flavonoid that can protect blood vessels. Some researchers
70 have stated that *T. cacao* can inhibit atherosclerosis through
71 modulation of oxidative stress and inflammation and nor-
72 malisation of blood pressure and blood lipid levels.²² Several
73 studies have suggested that *T. cacao* can inhibit the up-
74 regulation of vascular factors in pre-eclampsia, including
75 IL-6, sVCAM-1, and ET-1.²³ One *in silico* study proved that
76 *T. cacao* ingredients can interact with eNOS; this effect
77 suggests that *T. cacao* will also affect HSP90.²⁴ In addition,
78 flavanols from cacao may interfere with various disruptors
79 of NOS activity, such as NADPH oxidase and ADMA.²⁵
80 As far as we know, no studies have revealed a protective
81 effect of *T. cacao* on endothelial cells in pre-eclampsia
82 through HSP90 modulation and ADMA down-regulation.
83 Therefore, in this study, we implemented exposure of endo-
84 thelial cells to plasma of pre-eclamptic patients and evalu-
85 ated the effects of a *T. cacao* extract on HSP90 and ADMA.

86 Materials and Methods

87 Isolation and culture of human umbilical vein endothelial 88 cells (HUVECs)

89 This study was conducted *in vitro*. HUVECs that reached
90 the confluent state were subdivided into five groups: the
91 control group (no treatment), endothelial-cell group that was
92 exposed to 2% pre-eclamptic patients' plasma, and an
93 endothelial-cell group that was exposed to 2% pre-eclamptic
94 patients' plasma and treated with an ethanol extract of
95 *T. cacao* at various doses (25, 50, or 100 ppm). Isolation and
96 cultivation of HUVECs were carried out according to
97 methods detailed in other studies.^{23,24,26}

98 Isolation of plasma of pre-eclamptic patients

99 This procedure was carried out according to the method
100 described elsewhere.^{23,24,26} This procedure was approved by
101 the Health Research Ethical Committee, Faculty of
102 Medicine, Brawijaya University, Malang, Indonesia. Pre-
103 eclampsia plasma concentration of 2% is the optimal dose
104 that is capable of eliciting a response: a decrease in endothelial
105 relaxation of blood vessels according to another study.²⁷

106 Extraction

107 *T. cacao* beans were obtained from PTPN XII JI, Raja-
108 wali 49, Surabaya, East Java, Indonesia. Beans obtained in
109 dry form were the voucher of the Coffee and Cacao Research
110 Center, Jember, East Java, Indonesia. Extraction was carried
111 out by pulverizing the cocoa beans by 400 mesh grinding.
112 Next, 300 g of the powdered cacao beans was added to n-
113 hexane (1:3, w/v) and incubated for 48 h at room tempera-
114 ture. Then, the extract was filtered through Whatman paper
115 (size 40). The precipitate was oven-dried at a temperature of
116

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