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In vitro Exploration of Vasodilation Activity of the Methanol Extract of the Coptosapelta flavescens Korth stem

Khemasili Kosala*, Sjarif Ismail*, Ika Fikriah*, Agustina Rahayu Magdaleni*
*Faculty of Medicine, Mulawarman University/Universitas Mulawarman Samarinda
E-mail address: khemasili_k@yahoo.com; ismail8997@yahoo.com;

ABSTRACT

Introduction: *Coptosapelta flavescens Korth is a liana plant of the Rubiaceae genus. In East Kalimantan it is known as Akar Tambolekar or Merung. Its stem is ethnobotanically used as herbs to treat high blood pressure but it has not been scientifically proven yet.*
Method: *This study aims to prove the activity of Coptosapelta flavescens stem (BCF) extract as an antihypertensive by using a blood vessels isolated organ model through the action mechanism as a vasodilator. BCF was taken from Paser Regency, East Kalimantan Province. The extraction was macerated using methanol solvent, then concentrated using a vacuum rotavapour at the temperature of 50ºC. Vasodilator activity was tested using a 3 mm long rat aorta isolated organ with endothel put into Kreb's Henselheit solution at 37°C, pH 7.4 and fed by carbogen gas. The aorta is contracted using a phenylephrine solution, after the contraction reached its peak and plateaued, the extract solution or the extract's solvent solution (as control) was added cumulatively and the aortic tone activity was observed. The results were expressed in percent of aortic contractility.*
Results: *The results showed that BCF extract caused an increase in the reduction of aortic contractility percentage as extract concentration increase.*
Conclusion: *This proves that BCF's methanol extract can cause vasodilation in blood vessels with endothelium.*

Keyword*: Coptosapelta flavescens, antihypertensive, extract, isolated aorta, vasodilator*

INTRODUCTION

Coptosapelta flavescens Korth is a liana plant of the Rubiaceae genus. In East Kalimantan it is known as *Akar Tambolekar* or *Merung*, it is called *"Akar"* or root because the trunk is creeping on the ground. Ethnobotanically, it is used as a herb to treat high blood pressure, cough, shortness of breath [1] and also used as an aphrodisiac [2]. This research aims to prove the activity of methanol extract of Coptosapelta flavescens stem (BCF) as antihypertensive by using isolated organ model of rat aorta with endothelial through action mechanism as vasodilator [3; 4].

MATERIAL AND METHODS

Materials and Equipment

BCF was taken from Paser Regency of East Kalimantan Province. Its type identification was performed by the taxonomist of Faculty of Forestry of Mulawarman University. The stem was sorted and washed with running water then cut into small pieces and dried in an oven at 60°C until the water content became <10%, then ground into powder. Pro-analytic methanol for the extraction was purchased from Sigma-Aldrich

dealers. Ingredients for making Kreb's Henselheit solution such as NaCl, KCl, $MgSO_4$, Na_2HPO_4, KH_2PO_4, $CaCl_2$, glucose were purchased from reseller in Samarinda. Carbogen gas (95% O_2 and 5% CO_2) was purchased from gas suppliers in Samarinda.

Male rats aged 3-4 months weighing 200-250 gr were obtained from Pharmacology Laboratory of Medical Faculty (FK) of Mulawarman University Samarinda and the ethics approval for the animal testing has been given by the Medical Ethics Committee of FK Unmul.

The equipment used for the vasodilation test were: six-chambers isolated organ bath, octal bridge amplifier, Power Lab/16SP digital recorder, isometric transducer, pH meter, tweezers, surgical scissors.

Extraction

Extraction was by maceration using methanol solvent with a ratio of 1:4 for 5 days, then filtered and concentrated using vacuum rotavapor at 50ºC. Concentrated extract was then further dried by oven at 50ºC until its water content become <10%.

Rat Aorta Preparation

Wistar rats were anesthetised and then killed off by cervical dislocation, then their abdominal wall were opened by incision from the abdomen to the thoracic aorta. After pulmonary and cardiac resection, thoracic aortic dissection was performed from the inferior diaphragm to the aortic base. The aorta was cleansed carefully from the attached connective tissue so as not to damage the endothelium, and then the aorta was placed in a petri dish containing the carbogenated Kreb's Henselheit solution. The aorta is cut on the ring with a length of about 3mm. The aortic ring was then inserted into a 10 ml organ bath containing Kreb's-Henselheit solution pH 7.4 at 37°C and was flooded with carbogen gas. The installation of aortic ring was according to the method described by Ismail & Yuniati (2016) and Gonzales et al, (2015). After the aortic organ hook had been mounted on an isometric transducer, it was directly connected to the AD Instruments digital recorder amplifier with ChartV.5 program. The changes in aortic dilatation tone were recorded in grams in the computer.

Vasodilation Activity

Test prior to the treatment, the aortic ring was equilibrated for 90 minutes in Kreb's-Henselheit solution to stabilize it and the solution was replaced by a new Kreb's-Henselheit solution every 15 minutes. Once stabilized, it was tested with 10^{-3} M phenylephrine. If the aortic ring contracts as a response, then the smooth muscle was indeed intact. The aortic vasodilation activity test was performed only after the prepared aortic ring was shown to have its smooth muscle and endothelial layer intact. The aortic ring was then equilibrated first and measured with an isometric transducer. After reaching an equilibrium point, the aortic ring was first precontracted with 10^{-3} M phenylephrine until it reached peak contraction and plateaued. Subsequently, the methanol extract solution of *Coptosapelta flavecens* (EMBCF) and the extract's solvent as Control containing 10% DMSO was added to it with varying doses added cumulatively. The test was repeated five times [3], and the result of changes in smooth muscle tone was expressed in percentage of aortic tone. Percentage of aortic contractile tone is the value of aortic tone after administration of extract/Control minus aortic tone after phenylephrine administration then divided by the aortic tone after phenylephrine administration. A negative percentage of vascular contractile tone shows vasodilation activity in the blood vessels.

RESULTS AND DISCUSSION

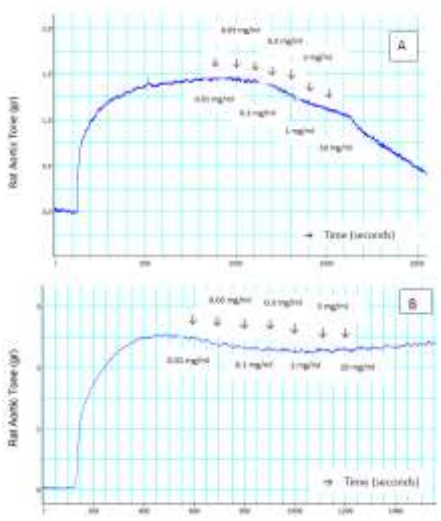

Figure 1. Vasodilatation of rat aortic ring after being given EMBCF (A) and the extract's solvent as Control (B) in cumulative concentration.
Note: Rat aortic ring in an organ bath containing Krebs solution after 90 minutes of equilibration. In the 100th sec, it was contracted with Phenylephrine until plateau, then the methanol extract of Coptosapelta flavecens stem (EMBCF) (A) and the extract's solvent (B) were given incrementally at 0.01 mg/ml, 0.03 mg/ml, 0.1 mg/ml, 0.3 mg/ml , 1 mg/ml, 3 mg/ml and 10 mg/ml with a time interval of 100 s.

Tabel 1. Percentage of aortic tones of rat with EMBCF

Log Kons mg/ml	Percentage of Aortic Tones			
	Controle		EMBCF	
	Mean	± SE	Mean	± SE
-2	-0,471	± 0,876	1,244	± 1,136
-1,5	-1,778	± 1,994	-4,921	± 3,707
-1	-2,963	± 2,924	-9,223*	± 3,614
-0,5	-3,464	± 2,553	-11,507*	± 4,580
0	-3,878	± 2,531	-14,366*	± 5,462
0,5	-4,981	± 2,327	-15,357*	± 4,324
1	-5,327	± 2,516	-16,289*	± 4,913

Note : n = 5, Control=Extract's solvent. EMBCF = Methanol Extracto of Coptosapelta flavescens Korth stem. Statistical test using t-test, significantly different if $p<0.05$. Extract with significantly different statistical test is marked with a star.

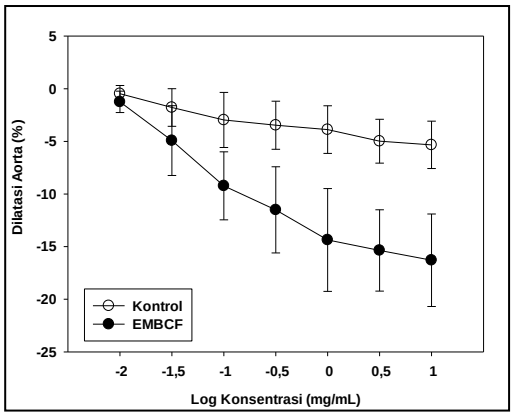

Fig 2. Dilatation Response Curve of Rat Aorta administered with Cumulative Doses of Methanol Extract of BCF
Note : n = 5, EMBCF = Methanol extract of Coptosapelta flavescens Korth stem, Statistical test using t-test, significantly different if $p<0.05$.

All data in Table 1 are the average ± SD of five repetitions (five rats). Table 1 shows the average yield of percent decrease of rat aortic tone after the cumulative administration of the methanol extract of Coptosapelta flavescens Korth stem (EMBCF), the extract's solvent containing DMSO 10% was used as controls. Dose Response Curve was obtained by plotting the concentration in semi-logarihmic scale to the percentage of aortic tone as shown in Figure 2. It is shown that as the concentration of Control and Extract given to isolated rat aorta with endothelium increases, the percent of aortic dilatation response is also increased. The aortic dilatation response appears to be stronger in the extract group compared with Control. This means that EMBCF contains secondary metabolites that have vasodilating activity in blood vessels with endothelium.

Previous studies have shown EMBCF contains polyphenols and saponin [5]. Several studies have proven polyphenols in green tea and saponin from *Panax notoginseng* shows vasodilation activity in the blood vessels. Lorenz et al. [6] proved that small concentrations of epigallocatechin-3-gallate polyphenols from green tea had strong vasodilatory activity in the aortic rings of wild-type rat, whereas vasodilation activity did not occur in eNOS-/- eliminated rats. Wang et al. [7] have proven that *Panax notoginseng*'s saponin and its five major components (ginsenosides Rg1, Re, Rb1, Rd and notoginsenoside R1) have vasodilatory activity in rat's aortic rings precontracted with nor epinephrine (NE).

EMBCF contains active ingredients such as polyphenols, saponin [5] which has been suspected able to activate specific endothelial receptors that cause endothelial nitric oxide synthase (eNOS) to produce NO [8]. NO is the main mediator that causes vasodilation by activating guanilic cyclase which produce cyclic guanosine-3,5-monophosphate (cGMP) and activates protein kinase G (PKG), which further decreases intracellular Ca^{2+} and causing relaxation [9]. In addition, the endothelial cells are also activated to produce Prostacyclin, which is a vasoactive substance, through the activity of cyclooxygenation enzyme (COX) from arachidonic acid which crossed the interstitial space and accumulates cyclic-AMP (cAMP) [7]. Other vasoactive substances produced by endothelial cells are endothelium-derived hyperpolarizing factors (EDHFs) that act as vasodilators by stimulating K^+ or Na^+-K^+ ATPase channels [10].

Besides having endothelial-dependent vascular vasodilator activity, the active ingredient in EMBCF may also have endothelial-independent vasodilator activity which needs to be further investigated in other studies.

CONCLUSION

The methanol extract of Coptosapelta flavescens stem showed a vasodilator activity *in vitro* on the Wistar rat aorta with endothel.

FUTURE DIRECTIONS

Further research is required in aorta without endothelium to show if the extract's vasodilators action mechanism is affected by endothelium or not for its further development as an antihypertensive herb.

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