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Antihypertensive and antioxidant activity of *Cassytha filiformis* L.: A correlative study

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ABSTRACT

Objective: To study the blood pressure lowering effect of *Cassytha filiformis* extract in animal models of hypertension and its correlation with the antioxidant activity.

Methods: Male Sprague–Dawley rats were divided into two groups: endocrine hypertension (HTN group) that received a combination of prednisone and salt for two weeks and oxidative stress-associated hypertension (HTN-OS group) that received additional induction of L-Nitro Arginine Methyl Esther (L-NAME) for two days. Each group was subdivided into 4 and treated intravenously with the extract 5; 10; and 20 mg/kg, and vehicle control. The systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) were recorded. The blood was taken before and at the end of recording for the measurement of serum concentration of nitric oxide (NO). The changes of blood pressure were analyzed by two-way ANOVA while its correlation with NO concentration was analyzed by Pearson's Correlation.

Results: The study showed a significant antihypertensive effect of the extract as compared with control group ($P < 0.05$) in both hypertensive models. Extract in the dose of 5 mg/kg showed the best blood pressure lowering effect. However, the correlation analysis did not show an association between NO increase and blood pressure lowering effect ($P > 0.05$).

Conclusions: The study concludes that *C. filiformis* extract in the dose of 5 mg/kg exhibits the best blood pressure lowering effect in both animal models. Antihypertensive activity of the extract is not correlated with its antioxidant effect.

1. Introduction

The pharmacological activities of *Cassytha filiformis* (*C. filiformis*) have gained attention over the past few decades. Studies concerning its chemical constituents have revealed that this herbaceous plant contains phenols, alkaloids, and flavonoids [1]. Some new chemical compounds with interesting pharmacological properties have been discovered [2]. The effects of these compounds are mainly within the cardiovascular system [3,4]. Other activities such as antiparasitic and cytotoxic have also been reported [5,6].

C. filiformis belongs to a large family Lauraceae with more than 2500 species distributed throughout tropical to subtropical latitudes. This family is well known for its pharmacological effects, especially in the cardiovascular system [7]. Our previous work has revealed the antihypertensive activity of *C. filiformis*. The blood pressure lowering effect of this plant tested to hypertensive rats was seen in a relatively small effective dose, 5 mg/kg [8].

Hypertension is one of most prevalent diseases worldwide estimated to cause about 12.8% of the total deaths [9]. One of the etiologies proposed for hypertension is oxidative stress due to excess level of oxidants over antioxidants. The oxidative stress may be caused by increased production of reactive oxygen species (ROS) or decreased level of nitric oxide (NO) [10]. ROS is believed to be responsible for the development of several cardiovascular diseases, such as atherosclerosis, hypertension, and congestive heart failure [11].

The antioxidant activity of *C. filiformis* has been recognized and considered as a potential source of antioxidant for the development of pharmaceuticals. The phenolic compounds present in the plant were thought to be responsible for its antioxidant properties [12,13]. The benefit of antioxidant supplementation had been extensively studied as an attempt to

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prevent and cure diseases in human [14,15]. These include aging-associated diseases [16], diabetes mellitus [17], and cardiovascular diseases [18].

There is no evidence whether the antihypertensive activity of *C. filiformis* is due and correlated to its antioxidant effect. Establishing a good and responsible correlation between the use of antioxidant compounds, especially phytochemical ones, and their prospective benefits in human is an important issue. Therefore, the present study was conducted to investigate how far the antioxidant content of *C. filiformis* may play a role for its blood pressure lowering activity in experimental models of hypertension. The study was also aimed to explore the correlation between the antioxidant effects of the plant, indicated by serum level of nitric oxide (NO), with its blood pressure lowering activity. The finding of this study is expected to give a relevant contribution on dose-effect consideration for the future development of this medicinal plant and herbal medicine in general.

2. Materials and methods

2.1. Drug and chemicals

Ethanol and *n*-hexane were purchased from Bratachem (Brataco PT, Indonesia). Prednisone was obtained from a local pharmacy, while heparin was produced by BBraun (Indonesia). L-Nitro Arginine Methyl Ester (L-NAME) was purchased from Sigma–Aldrich (USA), and nitric oxide colorimetric assay kit was purchased from BioVision Lab (USA).

2.2. Extract preparation

The whole part of *C. filiformis* plant used in this study was collected from Padang City, West Sumatra, Indonesia and identified in Herbarium of Andalas University (ANDA). The plant was dried at room temperature and protected from direct sunlight. The plant was cut into small pieces prior to extraction by dissolving it in sufficient amount of ethanol 96% for three days. The solvent was taken out and replaced with the new one to continue until complete extraction was achieved. The crude extract underwent further extraction with *n*-hexane to produce defatted extract and then concentrated.

2.3. Animal preparation

A total of 24 male Sprague–Dawley rats (weighing 250–300 g, aged 2–3 months) were acclimatized to normal laboratory condition for one week before experimental procedures. They were induced with daily oral prednisone-salt combination for two weeks to obtain animals with hypertension (HTN group). A half of the animals received additional L-NAME administration for 2 d to provoke hypertension associated with oxidative stress (HTN-OS group). The experimental protocol was approved by Ethics Committee of Faculty of Medicine, Andalas University No. 042/KEP/FK/2015.

2.4. Antihypertensive evaluation

The rats were anesthetized with pentobarbital 60 mg/kg administered intraperitoneally before surgical procedures. The carotid artery was cannulated for the direct BP measurement, while jugular vein was cannulated for extract administration and infusion. The rats also underwent tracheal cannulation to facilitate spontaneous respiration along with bladder catheterization for spontaneous urination. During the evaluation, the rats were infused with NaCl containing heparin 2 IU/mL and pentobarbital 2 mg/kg/h until the end of the procedure. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) were recorded using direct measurement method through the carotid artery by using Biopac MP150 (Biopac Data Acquisition System). The rats were divided into four groups: a control group and groups treated with 5, 10, and 20 mg/kg of extract. Each rat received three doses through intravenous administration with an hour interval [8].

2.5. Nitric oxide concentration measurement

An amount of 0.3 mL of blood was taken before and at the end of treatment through the carotid artery. The blood was centrifuged at 4000 rpm for 15 min to obtain the serum and stored at temperature −20 °C. All serum samples were prepared for the measurement of nitric oxide concentration (NO) through ELISA method by using spectrophotometer (BioRad).

2.6. Data analysis

Data of blood pressures (SBP, DBP, and MAP) were calculated as changes and analyzed by two-way ANOVA followed by Duncan's Multiple Range Test to evaluate the antihypertensive activity of the extract. The comparison of hypertensive level between HTN and HTN-OS was analyzed by Student's *t*-test. Meanwhile, the correlative analysis between MAP changes and NO increase was conducted by Pearson's correlation analysis. The significant level was taken at $P < 0.05$ for all statistical analysis.

3. Results

3.1. Prednisone-salt and prednisone-salt + L-NAME-induced hypertension

Both induction methods caused blood pressure elevation to reach hypertension level. The blood pressure of HTN-OS group showed a significant increase as compared with HTN group ($P < 0.05$). The elevation of SBP, DBP, and MAP from this group reached over 200 mmHg. The blood pressure elevation as the result of hypertensive induction of both groups is shown in Table 1.

Table 1
The elevation of systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) of the rats after hypertensive-induction in endocrine hypertension (HTN group) and oxidative stress-related hypertension (HTN-OS group).

Type of blood pressure	HTN group	HTN-OS group
Systolic Blood Pressure (SBP)	190.5 ± 3.61	229.2 ± 5.89
Diastolic Blood Pressure (DBP)	162.2 ± 3.55	204.1 ± 4.94
Mean Arterial Pressure (MAP)	176.5 ± 3.46	215.9 ± 5.21

Data are expressed as mean ± SEM; Unit: mmHg; $n = 12$.

Table 2

Blood pressure lowering effect of *Cassytha filiformis* extract in endocrine hypertension (HTN group) and oxidative stress-related hypertension (HTN-OS group).

Doses	SBP		DBP		MAP	
	HTN	HTN-OS	HTN	HTN-OS	HTN	HTN-OS
Control	6.0 ± 1.65^{q}	−7.0 ± 3.53^{q}	10.4 ± 1.75^{r}	−4.9 ± 3.67^{q}	8.6 ± 1.44^{r}	−5.7 ± 3.42^{q}
Extract 5 mg/kg	−16.4 ± 5.21^{p}	−44.7 ± 6.13^{p}	−20.9 ± 6.74^{p}	−40.8 ± 7.18^{p}	−18.4 ± 6.07^{p}	−42.3 ± 6.62^{p}
Extract 10 mg/kg	−6.8 ± 4.88^{pq}	−12.8 ± 3.94^{q}	−7.9 ± 6.12^{pq}	−14.0 ± 2.36^{q}	−7.4 ± 5.47^{pq}	−11.9 ± 2.73^{q}
Extract 20 mg/kg	−0.8 ± 4.71^{q}	−21.1 ± 4.73^{q}	2.4 ± 4.84^{qr}	−10.2 ± 4.41^{q}	0.6 ± 4.78^{qr}	−13.9 ± 4.32^{q}

Data are expressed as mean ± SEM; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; SEM: standard error of mean. Negative (−) values indicate the decrease of blood pressure. Different superscript within a column indicates significant difference (analyzed by two-way ANOVA with 95% confidence interval).

3.2. Antihypertensive activity of C. filiformis

The extract exhibited blood pressure lowering activity in both hypertensive animal models (HTN and HTN-OS groups). This antihypertensive effect was seen in all types of blood pressure (systolic, diastolic, and mean arterial pressure). The average decrease in HTN-OS group was significantly better as compared with that in HTN group (Table 2). The extract in the dose of 5 mg/kg showed the best lowering effect in both groups and was significantly different with control ($P < 0.05$). The blood pressure lowering effect of the extract in HTN and HTN-OS groups is shown in Figures 1 and 2.

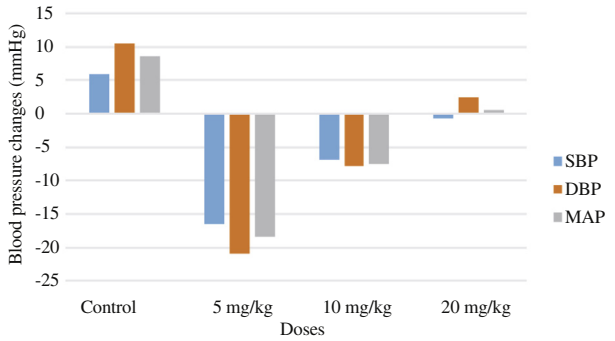

Figure 1. Blood pressure lowering effect of *Cassytha filiformis* extract in endocrine hypertension (HTN group) toward systolic (SBP), diastolic (DBP), and mean arterial pressure (MAP). Data with negative (−) values indicate the decrease of the parameters.

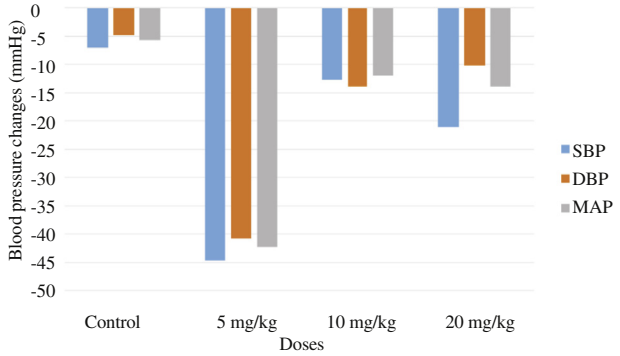

Figure 2. Blood pressure lowering effect of *Cassytha filiformis* extract in oxidative stress-related hypertension (HTN-OS group) toward systolic (SBP), diastolic (DBP), and mean arterial pressure (MAP). Data with negative (−) values indicate the decrease of the parameters.

3.3. Serum nitric oxide (NO) measurement

The result of serum nitric oxide (NO) concentration increase after the treatment of the extract is shown in Table 3. There is a marked increase of NO concentration due to the administration of the extract. The average increase of NO concentration is higher in HTN-OS group than HTN group. The biggest dose, 20 mg/kg, also caused the highest increase in serum NO concentration.

Table 3

The increase in serum nitric oxide (NO) concentration after the treatment of the extract in endocrine hypertension (HTN group) and oxidative stress-related hypertension (HTN-OS groups).

Doses	HTN group	HTN-OS group
Control	164.6	168.9
Extract 5 mg/kg	365.4	277.1
Extract 10 mg/kg	553.9	638.8
Extract 20 mg/kg	1042.7	1602.2

Unit: nmol/μL; $n = 12$.

3.4. Correlation analysis between blood pressure lowering effect and serum nitric oxide concentration

The blood pressure lowering effect of *C. filiformis* extract was not correlated with the serum concentration of nitric oxide ($P > 0.05$). The Pearson's correlation analysis did not confirm any correlation between SBP, DBP, and MAP with serum nitric oxide (NO) concentration in both HTN and HTN-OS groups. NO score in HTN group, r = 0.452, p = 0.222; NO score in HTN-OS group: r = 0.590, p = 0.094. The scatter plot of the correlation analysis is presented in Figure 3.

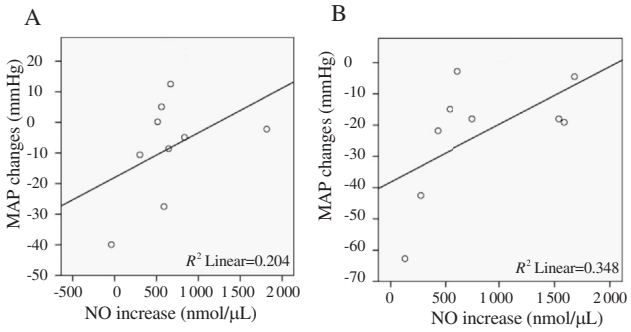

Figure 3. Scatter plots showing the correlation of the increase of serum nitric oxide (NO) concentration with the mean arterial pressure (MAP). Change in A) endocrine hypertension (HTN group), $R^2 = 0.204$; B) oxidative stress related hypertension (HTN-OS group), $R^2 = 0.348$.

4. Discussion

The present study occupied two different models of pharmacologically-induced hypertension: endocrine hypertension (HTN) and oxidative-stress related hypertension (HTN-OS). The combination of prednisone and salt is a modification of deoxycorticosterone acetate (DOCA)-salt model that has been used in our previous studies to obtain animals with high blood pressure [8]. This model represents the clinical situation of hypertension due to aldosterone excess [19]. This method has always been proven effective to increase the blood pressure of the rats to achieve the hypertensive level for the evaluation of antihypertensive activity of natural products. On the other hand, the latter group received additional L-NAME to obtain elevated blood pressure related to oxidative stress. L-NAME is commonly used to induce hypertension in experimental animals, mainly due to its oxidative stress effect [20,21]. The use of several different models in the evaluation of potential antihypertensive agents is strongly encouraged because the human hypertension is a complex and multifactorial condition [22].

The blood pressure measurement in the present study was carried out by direct measurement method through the carotid artery of the rats. This intra-arterial catheter method is considered more precise than others, despite invasive and requires surgery [23]. Nevertheless, the invasive blood pressure recording is suggested as the gold standard in experimental animal and is an essential part of the preliminary screening of any product to explore its pharmacological activities within the cardiovascular system [24].

The present study found that the blood pressure level of HTN-OS group was significantly higher than HTN group after two weeks of induction. This extra elevation was due to additional induction of L-NAME in HTN-OS group. L-NAME is a non-selective inhibitor of nitric oxide synthase, an enzyme that catalyzes the production of nitric oxide. This arginine analog is reported to impair NO signaling in arteries of the animals and cause its imbalance with ROS, thus leading to endothelial dysfunction and blood pressure elevation [25,26].

There was a significant positive antihypertensive activity of the extract in both animal models. This finding fits well with our previous study investigating the blood pressure lowering effect of the major fractions of the plant [8]. Besides rich in antioxidants [1,12,13], some vasorelaxing alkaloids and flavonoids have previously been reported from this plant [4]. However, the present study found that the average decrease in blood pressure in the oxidative-stress related group (HTN-OS) was significantly greater as compared with HTN group. This applies to all three types of blood pressures evaluated in the present study. The oxidative-stress related group was more responsive to the treatment, as hypothesized before. Lower antioxidant capacity in this group, due to extra induction with L-NAME, might cause the animal to be more responsive to the antioxidant contained in the extract [27].

All three doses of the extract showed a positive antihypertensive activity. Interestingly, the smallest dose, 5 mg/kg, exhibited the most effective blood pressure lowering effect and significantly better than other doses. This characteristic is possible and common in the use of antioxidant therapy. The use of antioxidants, especially in the area of cardiovascular system, may be beneficial but only when sufficient doses are used [27]. Some reports highlight ineffective antioxidants when used in greater dose, and sometimes associated with the incidence of side effects. Moreover, a large dose of antioxidant may increase the risk of certain cancers, as reported by a study in 2011 [28]. This phenomenon is probably related to the balance of antioxidant and prooxidant properties contained in the substance, especially in herbal products. Due to their prooxidant properties, and sometimes influenced by the technique used in the formulae preparation, the prooxidant hazardous effects of medicinal plants are recently gaining more attention beyond their beneficial effect [29]. The prooxidant properties can cause oxidative damage by reacting with various biomolecules, such as lipids, proteins, and DNA and causing undesired effects [30]. As a consequence, instead of possessing more effective blood pressure lowering effect, larger doses of the extract in the present study exhibited lower efficacy in reducing the blood pressure.

The correlation between serum level of nitric oxide and mean arterial pressure was tested by using Pearson's correlation method. The most remarkable result to emerge from the analysis is that the antihypertensive effect of the extract is not correlated with its antioxidant effect. The correlation was not confirmed in both endocrine and oxidative stress-related hypertensive groups. The study showed that the decrease in SBP, DBP, and MAP was not correlated with the increase of serum NO concentration after the extract administration. However, greater dose of the extract did cause a better increase of NO.

Antioxidants supplementation has been recommended due to their health benefit for the purpose of treating diseases in human. The idea of antioxidant use for high blood pressure has long been proposed, especially in the treatment purpose. However, this method remains challenging due to inconsistent results. Besides, the improvement of the disease is usually not seen, especially in those at high risk of cardiovascular diseases [31]. Sufficient evaluation is needed concerning the supplementation of antioxidants, especially for the purpose of primary or secondary prevention of diseases [32].

Conflict of interest statement

We declare that we have no conflict of interest concerning this study.

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