International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 2, 2016

Original Article

VASODILATATION EFFECT OF ETHANOLIC EXTRACT OF *ANREDERA CORDIFOLIA*, *SONCHUS ARVENSIS* L, AND URSOLIC ACID ON ISOLATED RABBIT AORTIC AND FROG HEART

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Received: 26 Oct 2015 Revised and Accepted: 12 Dec 2015

ABSTRACT

Objective: The objective of this study to investigate the vasodilation effect and the mechanism of ethanolic extract of *Anredera cordifolia, Sonchus arvensis* L and ursolic acid on isolated rabbit aortic and frog heart.

Methods: Aortic rings were placed in an organ bath and pre-contracted with Norepinephrine (2.9 x 10^{-3} mM) and potassium chloride (40µM) before addition of ethanolic extract of *Anredera cordifolia, Sonchus arvensis* L and ursolic acid. An anesthetized frog that exposes the heart was placed in organ bath and pre-contracted with Norepinephrine (2.9 x 10^{-3} mM). The vasodilation response by an extract of *Anredera cordifolia, Sonchus arvensis* L and ursolic acid were evaluated in the duration of contraction of aortic and a decrease of frequence and amplitude pattern of frog heart.

Results: Ethanolic extract of *Anredera cordifolia* (0.9 mg/ml) produced significant vasodilation of the norephineprine pre-contracted rabbit aortic rings ($p<0.05$) but not produced vasodilation of potassium chloride pre-contracted rabbit aortic rings. The vasodilation response to an ethanolic extract of *Anredera cordifolia* may be resulted through nitric oxide (NO), since the pretreatment of the isolated rabbit aortic rings with methylene blue inhibited the NO-mediated vasodilation. Moreover, the extract exhibited vasodilation of frog heart, which appeared may be mediated by inhibition of β1-adrenoreceptor. Whereas, ethanolic extract of *Sonchus arvensis* L. produced vasoconstriction of the norepinephrine and potassium chloride pre-contracted rabbit aortic rings. Afterward, continued for ursolic acid. The ursolic acid (0.5 µg/ml) not produced vasodilation effect of the norepinephrine and potassium chloride pre-contracted rabbit aortic rings, but exhibited vasodilation of frog heart; it means the ursolic acid had a role in vasodilation effect of frog heart of ethanolic extract of *Anredera cordifolia.* Moreover, this vasodilation effect may be mediated by inhibition of β1-adreno receptor, since the heart had β1-adrenoreceptor and inhibition of this receptor exhibited vasodilation (decrease frequency and amplitude). In addition, the pattern of vasodilation appeared to be similar with vasodilation induced by bisoprolol (2.5 µg/ml) and did not show as muscarinic agonist based on no vasodilation produced in isolated aortic.

Conclusion: The obtained results revealed that ethanolic extract of *Anredera cordifolia* exhibited vasodilation in rabbit aortic rings may be through facilitating the role of an endogenous compound such as nitric oxide (NO) and also exhibited vasodilation on frog heart may be mediated β1-adrenoreceptor inhibition. Whereas, the ethanolic extract of *Sonchus avensis* L produced vasoconstriction. In addition, ursolic acid compound not produced vasodilation effect of the norephineprine and potassium chloride pre-contracted rabbit aortic rings but exhibited vasodilation of frog heart that may be mediated β1-adrenoreceptor inhibition.

Keywords: *Anredera cordifolia, Sonchus avensis*, Ursolic Acid, Nitric Oxide, β1-adrenoreceptor.

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INTRODUCTION

Hypertension is the most common cardiovascular disease and impact 25-30% world population [1]. In a survey conducted in 2007/2008, hypertension was found in 29% of American adults. Moreover, based on riskesd as information in 2013, hypertension was found in 25.8% of Indonesia [2]. The prevalence varies according to age, race, education, and many other variables. According to some studies, 60–80% of both men and women will develop hypertension by age 80 [3]. Sustained arterial hypertension damages blood vessels in the kidney, heart, and brain and leads to an increased incidence of renal failure, coronary disease, heart failure, stroke, and dementia [4].

Effective drugs lowering of blood pressure have been shown to prevent damage to blood vessels and to reduce substantially morbidity and mortality rates [5,6]. Nevertheless, Indonesian people still believe in medicinal plants use to control hypertension or combined it with the drugs lowering of blood pressure. They have believed medicinal plants have more beneficial effects than their synthetic, counterpart through being safer, acceptable, affordable, and culturally compatible. Although in fact, many mechanisms of action that responsible for effect from the medicinal plants unknown [7]. *Anredera cardifolia and Sonchus arvensis* are an example medicinal plants that used to control hypertension by Indonesia people while ursolic acid is a major secondary metabolite in *Anredera cordifolia* that believed has the effect to reduce blood pressure [10, 11].

Understanding the mechanism of action that responsible for pharmacological effect is one of rationality consideration medication use. This study aimed to investigate the vasodilation effect and the mechanism of ethanolic extract of *Anredera cordifolia, Sonchus arvensis L* and ursolic acid on isolated rabbit aortic and frog heart.

MATERIALS AND METHODS

Materials

Kymograph (Harvard Apparatus/Universal Kymograph), Ethanolic extract of *Anredera cordifolia*, Ethanolic extract of *Sonchus arvensis* L, ursolic acid (Indogen), norepinephrine (Dexa Medica), potassium chloride, doxazosine (pfizer), nifedipine (kimia farma), bisoprolol (Hexpharm), methylene blue, dimethyl sulfoxide. All solutions were made fresh. All substances were dissolved in dimethyl sulfoxide. The final concentration of dimethyl sulfoxide never exceeded 0.9% (v/v) and this had no effect on the cell or assays. Except for norepinephrine, potassium chloride, bisoprolol and methylene blue, which were dissolved in aqua bidest.

Plant materials

Dried leaf of *Anredera cordifolia* and *Sonchus arvensis* was purchased from Manoko, Lembang, West Java, Indonesia and authenticated by School of Life Sciences and Technology Institute Technology Bandung, Indonesia.

Preparation of extract

Powdered crude of *Anredera cordifolia* leaves was extracted with ethanol 70% in the reflux apparatus. Whereas, powdered crude of *Sonchus avensis* leaves was extracted with ethanol 96% in the macerator apparatus. It was followed by evaporation using rotary evaporator until the viscous extract was obtained. This extract was referred as an ethanolic extract of *Anredera cordifolia* and *Sonchus avensis.* It was kept in refrigerator 4 °C until it would be used for pharmacological studies.

Methods

Aortic preparation

The experimental protocol was ethically approved by the ethical committee of the School of Pharmacy, Institute Technology Bandung. A rabbit of local strain (2.5-3.5 kg) was used in this experiment. The rabbit was murdered by carbon dioxide. The chest was opened. The internal viscera were pulled aside, and the aorta had been exposed. The aorta was cut close to the heart and dissected as far as possible. Then after, the tissue was transferred to a Petri dish containing a Krebs solution. Surrounding fats and connective tissues were removed and cut into rings 3 mm. Threads had been tied to each end of the rings and one end was attached to the tissue holder. The aortic in immersed condition using Krebs solution. The preparation was allowed to stand for 20 min, before addition of the reference drugs (Ethanolic extract of *Anredera cordifolia* (0.9 mg/ml), Ethanolic extract of *Sonchus avensis* L (0.9 mg/ml), ursolic acid (0.5µg/ml), doxazosin (4.5µg/ml), nifedipine, (9µg/ml).

Hearts frog preparation

The experimental protocol was ethically approved by the ethical committee at School of Pharmacy, Institut Teknologi Bandung. Expose the heart of pithed frog by cutting through the skin on the chest and through the pectoral girdle on both sides. Cut away the pericardium carefully. Place the frog on the corkboard mounted on a stand and passed a hook through the apex of the ventricle.

Config. the speed of kymograph. Record the normal pattern of the heart beats as a control and watches the heart rate and amplitude. Afterward expose the heart to norepinephrine (2.9 x 10^{-3} mM), after recording the effect of the drug on heart rate and amplitude of contraction, then expose the heart to reference drugs (Ethanolic extract of *Anredera cordifolia* (0.9 mg/ml), ursolic acid (0.5µg/ml) and bisoprolol (2.5µg/ml).

Statistical analysis

Statistical analysis was by one-way ANOVA followed by Least Significant Difference (LSD) post-hoc test by SPSS 16.0. The value of<0.05 was taken as a significant point.

RESULTS AND DISCUSSION

Norepinephrine induction

The effect of an Ethanolic extract of *Anredera cordifolia* and Ethanolic extract of *Sonchus avensis* L to the norepinephrine pre-contracted rabbit aortic rings were shown in table 1 below.

Table 1: Duration of contraction

Treatment	Duration of contraction (min)			
	1	2	3	Average
Norephineprine	104	99.84	110.24	104.69± 5.23
Norephineprine + Dimethyl sulfoxide	101.92	99.84	95.68	99.14± 3.17
Norephineprine + Doxazosin	10.4	18.72	18.72	15.94± 4.80*
Norephineprine + nifedipine	20.8	29.12	29.12	26.34± 4.80*
Norephineprine *+ Sonchus avensis*	Contraction	contraction	contraction	Contraction
Norephineprine *+ Anredera cordifolia*	56.16	58.24	56.16	56.85± 1.20*
	99.84	97.76	97.76	
Methylen blue + Norephineprine + *Anredera cordifolia*	56.16	58.24	56.16	98.45± 1.20

Values are expressed as mean±SD, * = significantly different from control group (p<0,05),

Based on the table above, ethanolic extract of *Sonchus avensis* L had vasoconstriction effect to the norepinephrine pre-contracted rabbit aortic rings. The ethanolic extract of *Anredera cordifolia* exhibited significant vasodilation effect (p<0.05) than control. This vasodilation response to an ethanolic extract of *Anredera cordifolia* may result through nitric oxide since the pretreatment of the isolated rabbit aortic rings with methylene blue not produced vasodilation or as same as with negative control (p>.0.05). The vasodilation is may via stimulation of muscarinic receptors (M3) that activate the G protein-phospholipase C-inositol triphosphate (Gq-PLC-IP3) pathway and mobilizes cell calcium. In endothelial cells, this leads to Ca2+-calmodulin dependent activation of endothelium nitric oxide synthetase (eNOS) and production of nitric oxide which diffuses to adjacent smooth muscle cells, where it stimulates the soluble guanylyl cyclase produced c-GMP and caused vasodilation or direct via NO itself that produced by secondary metabolite on the extract [8]. In Addition, the positive control group doxazosine and nifedipine exhibited significant vasodilation effect (p<0.05) than negative control and if we compared between doxazosine and nifedipine, doxazosine exhibited significant vasodilation effect (p<0.05) than nifedipine.

Potassium chloride induction

The effect of an Ethanolic extract of *Anredera cordifolia* and ethanolic extract of *Sonchus avensis* L to the potassium pre-contracted rabbit aortic rings were shown in table 2 below.

Table 2: Duration of contraction

Treatment	Duration of contraction (min)			
	1	2	3	Average
KCl	18.72	24.96	24.96	22.88± 3.60
KCl + Dimethyl sulfoxide	18.72	22.88	24.96	22.18± 3.17
KCl + Doxazosin	18.72	20.8	18.72	19.41± 1.20
KCl + Nifedipine	12.48	12.48	10.4	11.78± 1.20*
KCl + *Sonchus avensis*	contraction	contraction	contraction	Contraction
KCl + *Anredera cordifolia*	18.72	20.8	24.96	21.49± 3.17

Values are expressed as mean±SD, * = significantly different from control group (p<0,05),

Based on the table above, ethanolic extract of *Sonchus avensis* L had vasoconstriction effect on the norepinephrine pre-contracted rabbit aortic rings. The ethanolic extract of *Anredera cordifolia* not exhibited vasodilation effect and showed time duration of contraction as same as negative control group.

This result indicated if an ethanolic extract of *Sonchus avensis* did not have inhibition in calcium channel. In Addition, the positive control group doxazosin and nifedipine exhibited significant vasodilation effect ($p<0.05$) than negative control and if we compared between doxazosin and nifedipine, nifedipine exhibited significant vasodilation effect ($p<0.05$) than doxazosin. This result indicated that potassium chloride-induced calcium channel to open. Potassium chloride has long been used as a convenient stimulus to bypass G protein-coupled receptors (GPCR) and activate smooth muscle by a highly reproducible and relatively "simple" mechanism involving activation of voltage-operated Ca2+channels that leads to increases in cytosolic free Ca2+([Ca2+]i), Ca2+-calmodulin-dependent myosin light chain (MLC) kinase activation, MLC phosphorylation and contraction[12].

Frog heart

The effects of an Ethanolic extract of *Anredera cordifolia* to the norepinephrine pre-contracted heart frog were shown in fig. below.

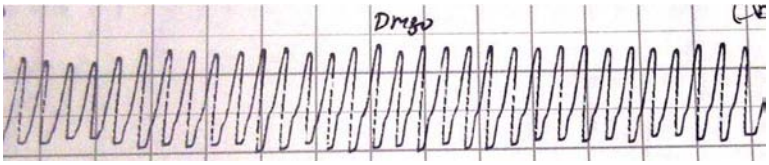

Fig. 1: Contraction after treated with NE followed by dimethyl sulfoxide

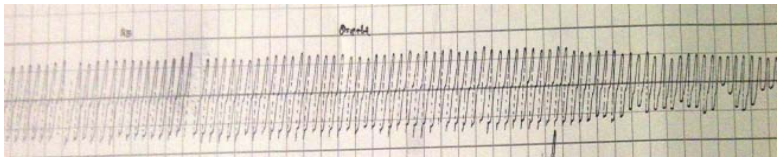

Fig. 2: Contraction after treated with NE followed by bisoprolol

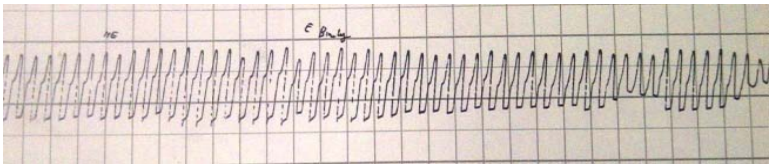

Fig. 3: Contraction after treated with NE followed by Anredera cordifolia extract

Based on the fig. above ethanolic extract of *Anredera cordifolia* exhibited a decrease in heart rate and amplitude of the heart as same as with bisoprolol. This vasodilation of frog heart may be mediated by inhibition of β1-adrenoreceptor, since the β1-adrenoreceptor concentrate in the heart, it is referred to as a beta-adrenergic organ and inhibition of this receptor exhibited vasodilation characterized with decrease frequency and amplitude [13]. In addition, the pattern of vasodilation appeared to be similar with vasodilation induced by bisoprolol (2,5 µg/ml) or mediated by the muscarinic receptor. Whereas, Dimethyl sulfoxide did not change heart rate and amplitude of the heart.

Norepinephrine induction

To proved what is the responsible for the vasodilation effect in ethanolic extract of *Anredera cordifolia,* we continued for the ursolic acid test (secondary metabolite of *Anredera cordifolia*). The effects of ursolic acid to the norepinephrine pre-contracted rabbit aortic rings were shown in table 3 below.

Table 3: Duration of contraction

Treatment	Duration of contraction (min)			
	1	2	3	Average
Norephineprine	131.04	122.72	114.4	122.72± 8.32
Norephineprine + Dimethyl sulfoxide	128.96	124.8	110.24	121.33± 9.82
Norephineprine + Doxazosin	18.72	12.48	18.72	16.64± 3.60*
Norephineprine + Nifedipine	39.52	43.68	43.68	42.29± 2.40*
Norephineprine + Ursolic Acid	128.96	120.64	116.48	122.02± 6.35

Values are expressed as mean±SD, * = significantly different from control group ($p<0,05$),

Based on the table above, ursolic acid not exhibited vasodilation effect and showed the duration of contraction as same as a negative control group. This results indicated if ursolic acid did not have in αlpha-1 inhibition and muscarinic agonist, since the norepinephrine as an agonist αlpha-1 and. In Addition, the positive control group doxazosin and nifedipine exhibited significant vasodilation effect ($p<0.05$) than negative control and if we compared between doxazosin and nifedipine, doxazosine exhibited significant vasodilation effect ($p<0.05$) than nifedipine.

Potassium chloride induction

The effects of ursolic acid to the potassium chloride pre-contracted rabbit aortic rings were shown in table 4 below.

Table 4: Duration of contraction

Treatment	Duration of contraction (min)			
	1	2	3	Average
KCl	27.04	27.04	31.2	28.42 ± 2.40
KCl + Dimethyl sulfoxide	27.04	29.12	24.96	27.04 ± 2.08
KCl + Doxazosin	18.72	18.72	22.88	20.10 ± 2.40*
KCl + Nifedipine	14.56	12.48	12.48	13.17 ± 1.20*
KCl + Ursolic Acid	27.04	29.12	29.12	28.42 ± 1.20

Values are expressed as mean±SD, * = significantly different from control group (p<0,05),

Based on the table above, ursolic acid not exhibited vasodilation effect and showed the duration of contraction as same as a negative control group (p>0.05). This result indicated if ursolic acid did not have calcium channel inhibition since potassium chloride has a function to open the calcium channel causes the influx of calcium [12]. In Addition, the positive control group doxazosin and nifedipine exhibited significant vasodilation effect (p<0.05) than the negative control.

Frog heart

The effect of ursolic acid to the norepinephrine pre-contracted heart frog was shown in the picture below.

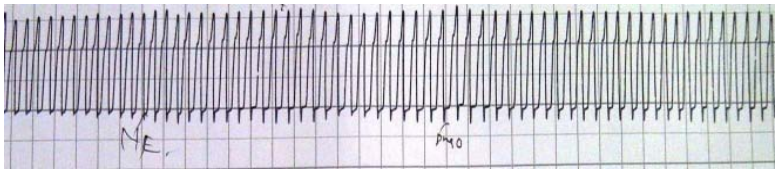

Fig. 4: Contraction after treated with NE followed by dimethyl sulfoxide

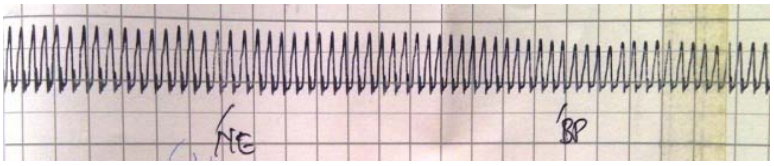

Fig. 5: Contraction after treated with NE followed by bisoprolol

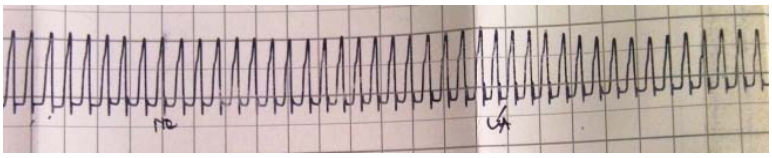

Fig. 6: Contraction after treated with NE followed by ursolic acid

Based on the picture above ursolic acid exhibited a decrease in heart rate and amplitude of the heart as same as with bisoprolol. This indicated if the ursolic acid had a role in vasodilation effect of frog heart of ethanolic extract of *Anredera cordifolia.* Moreover, this vasodilation effect ursolic acid may be mediated by inhibition of β1-adrenoreceptor since the heart had β1-adrenoreceptor and inhibition of this receptor exhibited vasodilation characterized with decrease frequency and amplitude [13]. In addition pattern of vasodilation appeared to be similar with vasodilation induced by bisoprolol (2, 5 µg/ml) and did not show as muscarinic agonist based on not exhibited vasodilation produced in isolated aortic. Whereas, Dimethyl sulfoxide did not change heart rate and amplitude of the heart.

CONCLUSION

Based on experiment evidenced, it can be concluded if the ethanolic extract of *Anredera cordifolia* exhibited vasodilatation that may mediate by the role of nitric oxide (NO). Moreover, this extract exhibited vasodilation in frog heart characterized by a decrease of heart rate and amplitude. This vasodilatation may mediate by β1-adrenoreceptor inhibition. Nevertheless, ethanolic extract of *Sonchus avensis* L. conversely produced vasoconstriction of the aortic (amplified of norepinephrine). While, the result for ursolic acid as one of a secondary metabolite of ethanolic extract of *Anredera cordifolia* not produced vasodilation effect of the norepinephrine and potassium chloride pre-contracted rabbit aortic rings and show as same as a negative control group, but this ursolic acid exhibited vasodilation of frog heart that may mediate by β1-adrenoreceptor inhibition. This resulted indicated the ursolic acid had a role and responsibility in vasodilation effect of frog heart of ethanolic extract of *Anredera cordifolia.*

CONFLICT OF INTERESTS

All authors have none to declare.

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