Research Article

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VASODILATION EFFECT OF OLEANOLIC ACID AND APIGENIN AS A METABOLITE COMPOUND OF *ANREDERA CORDIFOLIA (TEN) V. STEENIS* ON ISOLATED RABBIT AORTIC AND FROG HEART

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ABSTRACT

Apigenin and oleanolic acid are secondary metabolite of *Anredera cordifolia (Ten) V.Steenis*. The plants are also recognized to have antihypertensive activities. Vasodilation is a major factor of cardiovascular function. The aim of the study is to determine the vasodilation and the mechanism of action of oleanolic acid and apigenin. The aortic rings were placed in an organ bath and precontracted with norephineprine (2.9 x 10^{-3} mM), methylene blue followed by norephineprine (2.9 x 10^{-3} mM) and potassium chloride (40µM) for apart before addition of oleanolic acid (0.5µg/ml) and apigenin (0.05µg/ml). Anesthetized frog that exposed the heart was placed in an organ bath and precontracted with norephineprine (2.9 x 10^{-3} mM) before addition of oleanolic acid (0.5µg/ml) and apigenin (0.05µg/ml). The vasodilation response in isolated rabbit aortic was evaluated through time duration to reach 100% relaxation and decreased of the heartbeat of the frog heart. Oleanolic acid (0.5µg/ml) produced significant vasodilation of the norephineprine pre-contracted rabbit aortic rings ($p<0.05$) but not with methylene blue followed with norephineprine. In addition, oleanolic acid not produced vasodilation with pre-contracted of potassium chloride. Moreover, oleanolic acid exhibited vasodilation of frog heart. While, for the apigenin (0.05µg/ml), it was produced vasodilation effect of the norephineprine, methylene blue followed by norephineprine and potassium chloride pre-contracted rabbit aortic rings ($p<0.05$). Moreover, apigenin exhibited vasodilation of frog heart. In conclusion, oleanolic acid exhibited vasodilation through facilitating of nitric oxide (NO) and β1-adrenoreceptor inhibition. Whereas, the apigenin exhibited vasodilation effect through facilitating inhibition of calcium channel and β1-adrenoreceptor inhibition.

Keywords: Oleanolic acid, Apigenin, Nitric oxide, Calcium channel blocker, β1-adrenoreceptor

INTRODUCTION

Blood pressure is calculated as cardiac output time peripheral resistance. Rise of Blood pressure called as a Hypertension. Hypertension is one cause of cardiovascular disorder and death. Various scattered references are present in Ayurvedic literature depicting Hypertension disease[1]. Hypertension is characterized by a rise of blood pressure, which in turn damage many of the body system, in particular blood vessel and any organ target[2]. Controlled of blood pressure showed decrease the risk of cardiovascular disease [3]. Apart from currently available therapeutic options, various herbal medicines have made recommendation for the treatment of hypertension. Traditional plant medicines are utilized throughout the world for a range of hypertension presentations. Indonesia is a country that richness of medicinal plants. *Anredera cordifolia (Ten) V. Steenis* is one of the medicinal plants that have been used to control hypertension by Indonesian people [4-5].

In hypertensive patient, vasodilation of the blood vessel is an important factor in protection and alleviate of blood vessel resistance and heart load , that finally reduce the blood pressure. Ethanolic extract of *Anredera cordifolia (Ten) V. Steenis* and one of the active compound namely ursolic acid was proved to have vasodilation effect [6].Besides of ursolic acid, another active compounds found in *Anredera cordifolia* (Ten) V. Steenis are apigenin and oleanolic acid that are thought having the effect to reduce blood pressure [7,8]. Therefore, the study aimed to determine the vasodilation effect, the mechanism of action and continuation of the experiment previously.

MATERIALS AND METHODS

Kymograph (Harvard Apparatus/Universal Kymograph), oleanolic acid were obtained from tokyo chemical industry co.,ltd and apigenin were obtained from Santa cruz Biotechnology. Norephineprine (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 1% (v/v) except for norephineprine, bisoprolol, potassium chloride, and methylene blue dissolved in aquadest. The final drug concentrations tested were as follows: oleanolic acid 0.5 µg/mL and apigenin 0.05 µg/mL

The experimental protocol was ethically approved by the ethical committee of School of Pharmacy, Institut Teknologi Bandung with approval number of 01/KEPHP-ITB/11-2015. The handling the animal was carried out as per good laboratory practice (GLP) guidelines.

Animal

The male white rabbit and frog (bufo melanostictus) obtained from animal husbandry, Tasikmalaya. Before the experiments the, rabbit and frogs were fed with a standard diet. Before initiation of experiments, rabbits and frog were acclimatized for 5 days under standard environmental conditions of temperature, relative humidity and dark/light cycle.

Aortic preparation

The male white rabbit was employed in this experiment. The rabbit was sacrificed. The chest was opened. The internal viscera was pulled aside and the aorta had been exposed. The aorta was cut closed 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 only were removed and cut into rings 3mm. 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 minutes, before addition of the pre-contracted and reference drugs (oleanolic acid (0.5µg/ml) and apigenin (0.05µg/ml)

Hearts frog preparation

The heart of anesthetized frog was exposed by cutting through the skin on the chest and through the pectoral girdle on both sides. Cut away the pericardium carefully. The frog was placed on the cork board mounted on a stand and pass a hook through the apex of the ventricle. Configure the speed of kymograph. The normal pattern of the heart beats was recorded as a control and the heart rate and amplitude was observed. Afterward the heart was exposed to norephineprine (2.9 x 10^{-3} mM), after recording the effect of the drug on heart rate and amplitude of contraction, then the heart was exposed to reference drugs (oleanolic acid (0.5µg/ml) and apigenin (0.05µg/ml).

Statistical analysis

Statistical analysis were 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 significant point.

RESULTS AND DISCUSSION
Norephineprine induction

The effect of oleanolic acid and apigenin to the norephineprine pre-contracted rabbit aortic rings were shown in table 1 below.

Table 1: **Duration of contraction**

Treatment	Average (minute)
Norephineprine	104,69 ± 5,23
Norephineprine + Dimethylsuphoxide	99,15 ± 3,18
Norephineprine + Doxazosin	15,95 ± 4,80*
Norephineprine + Nifedipine	26,35 ± 4,80*
Norephineprine + Apigenin	67,9 ± 13,53*
Norephineprine + Oleanolic acid	42,99 ± 7,87*
Methylen.Blue + Norephineprine + Apigenin	62,40 ± 5,50*
Methylen Blue + Norephineprine + Oleanolic acid	103,31 ± 5,23

Experiments were carried out in triplicate and results are expressed as means of three replicate experiments. * = significantly different from control group (p < 0,05).

Based on the table above dimethylsulphoxide didn't have any effect of the aortic rings. This result indicated if dimethylsulphoxide as a solvent is safe for the experiment. The oleanolic acid produced vasodilation in pre-contracted norephineprine ($p<0.05$) but didn't produce vasodilation ($p>0.05$) in existence of methylene blue (guanilyl cyclase inhibitor). This result indicated if the vasodilation of oleanolic acid mediated by nitric oxide, since the pretreatment of the isolated rabbit aortic rings with methylene blue didn't produce vasodilation. The inhibition of vasodilation effect of oleanolic acid in existence of methylene blue because of inhibition of gualilyl cyclase, and this inhibition result inactivation of this enzyme. The inactivation of the enzyme inhibit interaction with nitric oxide that produced c-GMP as a vasodilator [9].While, for the apigenin produced vasodilation in pre-contracted norephineprine as well as in existence of methylene blue ($p<0.05$). This result indicated if the vasodilation of apigenin not mediated by nitric oxide, but may through inhibition of alpha receptor or calcium channel.

Potassium chloride induction

The effect of oleanolic acid and apigenin to the potassium chloride pre-contracted rabbit aortic rings were shown in table 2 below.

Table 2: Duration of contraction

Treatment	Average (minute)
Potassium Chloride	31,20±2,08
Potassium Chloride + Dimethylsuphoxide	31,89±7,30
Potassium Chloride + Doxazosin	29,81±2,40
Potassium Chloride + Nifedipine	14,56±2,08*
Potassium Chloride + Apigenin	14,56±2,08*
Potassium Chloride + Oleanolic Acid	30,51±1,20

Experiments were carried out in triplicate and results are expressed as means of three replicate experiments. * = significantly different from control group (p < 0,05).

Based on potassium chloride pre-contracted, apigenin produce vasodilation ($p<0.05$) as well as nifedipine. This result indicated and confirmed if vasodilation effect of apigenin through Calcium Channel Inhibition, since 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 [10]. Neverthless, oleanolic acid didn't produce vasodilation in potassium chloride pre-contracted. This indicated and confirmed if vasodilation of oleanolic acid through nitric oxide.

Frog heart

The effects of oleanolic acid and apigenin to the norephineprine pre-contracted heart frog were shown in figure below.

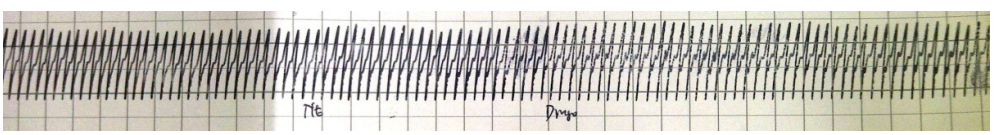

Figure 1: **Norephineprine induction followed by dimethylsufoxide**

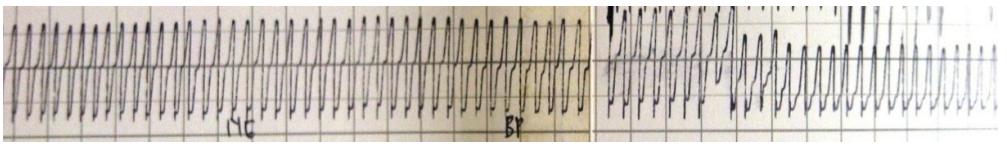

Figure 2: Norephineprine induction followed by bisoprolol

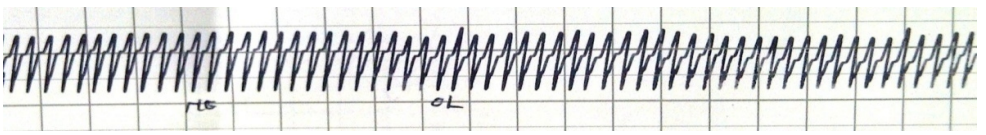

Figure 3: Norephineprine induction followed by oleanolic acid

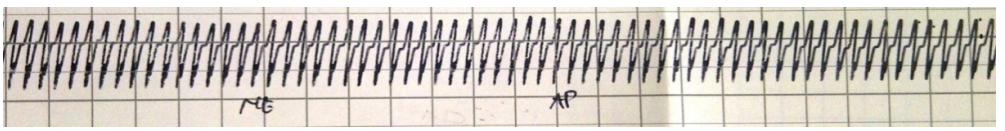

Figure 4: **Norephineprine induction followed by apigenin**

The figure 3 and 4 showed oleanolic acid and apigenin produced decreased of the heartbeat in frog heart through β1-adrenoreceptor inhibition, since the heart is β1-adrenoreceptor organ and can be used for examining selective activity to the receptor contained [11]. All the results in aortic rabbit and frog heart experiment indicated if apigenin and oleanolic acid as a metabolite compound of *Anredera cordifolia (Ten) V. Steenis* have an important role in hypertensive effect of *Anredera cordifolia (Ten) V. Steenis*.

CONCLUSION

The experiment conducted as a sequel of a previous experiment of *Anredera cordifolia (Ten) V. Steenis* effect. Oleanolic acid produced vasodilatation effect through the role of nitric oxide and inhibition of β1-adrenoreceptor. Whereas apigenin produced vasodilation effect through calcium channel inhibition and β1-adrenoreceptor inhibition. This resulted indicated the oleanolic acid and apigenin have an important role and may simultaneously responsible in antihypertension of *Anredera cordifolia (Ten) V. Steenis*.

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