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RESEARCH ARTICLE

The Effect of *Thymus Vulgaris* Extract on Smooth Muscle Relaxation of Guinea Pig Aortic Ring: Study *In Vitro*

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Abstract

Objective: Hypertension appears to have a complex association with endothelial dysfunction. One of herbs that potential to develop as anti hypertension is *Thymus vulgaris* which contains high concentrations of phenols, including thymol and carvacrol that offer protection from hypertension. This study aimed to prove the correlation between different concentration of *Thymus vulgaris* extract and response of aortic ring relaxation. Methods: Using a laboratory experiment (in vitro study), treatment with provision doses of *Thymus vulgaris* extract was conducted to guinea pig (aorta ring) after pre-contracted by phenylephrine (PE). *Thymus vulgaris* extract (TM) at the doses of 0.0625, 0.125, 0.25, 0.5 and 1% were administered to experimental groups including 5 doses of extract with intact aorta rings and 5 doses of extract with denuded aorta rings. Pearson correlation test and T-test were used to analyze the data. Results: The results indicate that administration of *Thymus vulgaris* extract can decrease the contraction of aorta guinea pig. Different doses of *Thymus vulgaris* extract have different relaxation responses of smooth muscle cell in aortic rings of guinea pig. The lower concentrations doses of *Thymus vulgaris extract* (0.0625% and 0.125%) can cause an effective and better relaxation effect on contraction of smooth muscle in denuded aorta than in intact aorta. Conclusion: The higher concentrations doses of *Thymus vulgaris extract* (0.025%, 0.5% and 1%) can increase the contraction of smooth muscle for both intact and denuded aorta of guinea pig.

Keywords: *Thymus vulgaris, intact aorta rings, denuded aorta rings, phenylephrine and smooth muscle cells.*

Introduction

Hypertension is defined as either a sustained systolic blood pressure of greater than 140mm Hg or a sustained diastolic blood pressure of greater than 90mm Hg. It results from increased peripheral vascular arteriolar smooth muscle tone, which leads to increased arteriolar resistance and reduced capacity of the venous system [1, 2].

Hypertension appears to have a complex association with endothelial dysfunction, a phenotypical alteration of the vascular endothelium that precedes the development of adverse cardiovascular events and portends future cardiovascular risk [3]. Furthermore, a damage to vascular endothelium due to atherosclerotic processes of the following ischemia and reperfusion alters the information also, release of endothelial factors. When endothelial damage occurs, the endothelium produces nitric oxide prostacyclin, which causes the adrenergic vasoconstrictor tone to be unopposed. This can affect to increased vascular tone and vasospasm.

Furthermore, decreased production of these endothelial factors can lead to increased platelet adhesion, aggregation, and enhanced thrombogenesis [1]. The endothelium exerts a number of vasoprotective effects, such as vasodilation, suppression of smooth muscle cell growth, and inhibition of inflammatory responses [4].

There are varieties of medications used to treat high blood pressure. These drugs are called anti hypertension agents. Partly most medicine of hypertension in Indonesia contains active substance form synthetic compounds, such as bromhexin, ambroxol, ammonium chloride and glyceryl guaiakolat. Nonetheless, these ingredients can cause side effects. Obviously, Nitric oxide (NO) and endothelial cells (EC) mechanisms involved in the pathogenesis of hypertension. However, there are many other herbs/spices that appear to have significant effects in favorably modulating high blood pressure.

Globally, the use of herbal therapies to treat and manage cardiovascular disease (CVD) is on the rise [5]. One of herbs that potential to develop as anti hypertension is *Thymus vulgaris*. Approximately 150 species of *Thymus* are abundantly found, mainly in Asia, Africa, and North America. Recently, its range has been widely been extended to the Iberian Peninsula, with most of the species being endemic [6]. Thyme contains high concentrations of phenols, including thymol (12-61%), carvacrol (0.4-20.6%), 1, 8-cineole (0.2-14.2%), q-cymene (9.1–22.2%), linalool (2.2-4.8%), borneol (0.6-7.5%), a-pinene (0.9-6.6%), and camphor (0-7.3%). Carvacrol and thymol are the main phenolic components that are primarily responsible for its antioxidative activity [7].

In addition, thyme oil is widely used in phytotherapy, most notably to treat and offer protection from acne, hypertension, infections, and cancers [8]. The oil contains bioactive monoterpenes such as thymol, carvacrol, and linalool [9]. Thus, this study was intended to explore the effect of *Thymus vulgaris* extract on vascular function. It focused on finding out the acts of the *Thymus vulgaris* extract on α1-adrenoceptor and endothelial cells of smooth muscle cells of Guinea pig blood vessels and in proving the correlation between different concentration of *Thymus vulgaris* extract and response of aortic ring relaxation.

Methods

Location and Time Study

The research was conducted at Pharmacology Laboratory of Brawijaya University in Malang. This study was conducted from July 2018 to August 2018. The result of the experiments was discussed in the report writing that was done in the period of time started from May 2018 to February 2019.

Thymus Vulgaris Extraction and Evaporation

Thymus Vulgaris leaves, flowers and stems (whole the plant) were obtained from local market (herbalist) in Msallata-Libya. The extraction method used maceration was conducted in Pharmacology Laboratory Faculty of Medicine, Universitas Brawijaya. A sample of 100 g of the herb was rinsed, chopped into small pieces and dried in an oven at a temperature of 80°C. The dried herb was ground.

The powder was soaked and shaked into 98% ethanol in I L erlenmeyer tube until the volume reach 1000 mL for over night. Supernatan was filrered and pour in rotary evaporator tube. After setting up the equipment, distilled water was heated until 70 °C to evaporate the solvent (ethanol) for about 2 hours. The crude extract was gain when the solvent fully evaporated.

Treatment Groups

The study was conducted using a laboratory experiment (in vitro study) and performed with simple purposive sampling. Treatment with provision doses of *Thymus vulgaris* extract was conducted to guinea pig (aorta ring) after pre-contracted by phenylephrine (PE). *Thymus vulgaris* extract (TM) at the doses of 0.0625, 0.125, 0.25, 0.5 and 1% were administered. Experimental design was conducted in the treatment groups (5 doses of extract with intact aorta rings and 5 doses of extract with denuded aorta rings).

Preparation of Aorta

The aorta ring with and without endothelium cell of healthy adult male guinea pig were prepared, because female guinea pig has higher estrogen hormone that could influence the result. To prepare the isolated aorta with endothelium (Intact aorta), the guinea pig was anaesthetize then dissects the guinea pig's thorax. Descending aorta was taken quickly by slicing fascia at lateral aorta with scalpel, and then proximal part of aorta was cut as soon as possible at the end of aorta arch and distal on mediastinum. The aorta was put quickly into petri dish that contain Kreb's (physiologic) solution. Aorta was cleaned from connective tissue and blood cloth then cut into ring form with length 4-5 mm.

Denuded aorta was rubbed with stainless steel hook in lumen blood vessel. Each ring was then suspended under a resting tension of 1 g in an organ bath containing Krebs solution in 37°C and pH 7.2-7.4 and connected to the isometric transducer to convert the mechanic to electric signal the convert to visual signal in the monitor.The aorta was equilibrate/adaptated to the in vitro condition about 1-2 hours until the contractility was stable. The contractility recorded and appeared in monitor on the power lab. During the stabilization, washing out was done by replacing Krebs solution for each 15 minutes.

Preparation and Dillution of Drug Stock

Phenylephrine (PE) was prepared by diluting in distilled water for concentrations PE10^{-2} M, and then diluted to concentrations 10^{-3} M, 10^{-4} M, 10^{-5} M, 10^{-6} M and 10^{-7} M. The acetylcholine (ACh) was prepared at a concentration $^{-5}$ M and Isosorbide dinitrate (ISDN) was prepared by taking a concentration dose at ISDN10^{-2} M and then diluted to doses 10^{-3} M, 10^{-4} M, 10^{-5} M and 10^{-6} M to compared the relaxation od the aorta by anti-hypertension drug.

Treatment Description

Treatment was started when the contractility of aorta reached a steady state. The treatments was as follows: 1) Administration of 10^{-6} M PE 0.1 ml to aorta ring in 10 ml Kreb's solution on organ bath. It was administered from a lower to a higher concentration: PE10^{-0} M, PE10^{-8} M, PE10^{-7} M, PE10^{-6} M, PE10^{-5} M, and PE10^{-4} M (administered if the contravtility reached steadt state), followed by washing several times with Kreb's solution.

The contraction effects of all concentrations were recorded by power lab to find out the dose response curve; 2) secons step was giving 0.1 ml of PE10^{-4} M to precontracted aorta ring in organ bath until the contractility stable, then Isosorbide dinitrate (ISDN) 0.1ml was added to aorta ring in organ bath with Kreb's solution 10 ml, starting from a lower concentration to a higher concentration: 10^{-6} M, 10^{-5} M, 10^{-4} M, 10^{-3} M, and ISDN10^{-2} M, followed by washing several times with Kreb's solution. The relaxation responses of all concentrations were recorded by PowerLab to developed the standard curve; 3) giving acetylcholine concentration Ach 10^{-5} M with volume 0.1 ml in aorta ring in organ bath after pre-contracted by PE 10^{-4} M, followed by washing out several times with Kreb's solution and the effect of ACH on aorta ring was recorded to know if aorta ring was intact or denuded; and 4) giving PE 10^{-4} M with volume 0.1 ml in aorta organ bath. After the maximal contraction was obtained, the administration of *Thymus vulgaris* was conducted from the lowest concentration of TM 0.0625% to higher doses of TM 0.125%, 0.25% ,0.5%and 1%. The responses were recorded by PowerLab. This last step was performed to intact aorta and denuded aorta groups with three times repetition.

Data Collection Procedure

The data collection procedures started by measuring the effect of the extract using PowerLab apparatus. PowerLab has capability to convert from mechanical contractility to electric signal by isometric transducer, then the electric signal amplified to the visual signal and the result was graph. considerable computing power of its own and performs many tasks during data recording. Once the PowerLab transfers data to the computer, it is available for display, manipulation/tratments, printing, storage and retrieval. The PowerLab 4/30 has four inputs for recording external signals, the 8/30 has eight and the 16/30 has sixteen [10].

Data Analysis

The results data from this research was analyzed statistically with one way ANOVA at significance P <0.05 using SPSS 16.0. Pearson correlation test was performed to find out the significant relationship between different concentration of *Thymus vulgaris* extract with relaxation effect on aorta after precontracted by phenylpherine. It was followed by a T-test to compare the effects of *Thymus vulgaris* concentration in two groups (intact and denuded).

Result and Discussion

Treatment of Thymus Vulgaris Extract on the Contractility of Intact and Denuded Aorta of Guinea Pig

This study was done in order to know the response of intact aorta contractility, after the administration of different concentration of *Thymus vulgaris* (TM 0.0625%, TM 0.125%, TM 0.25%, TM 0.5% and TM 1%). The PE 10^{-4}M was used to stimulate the aorta contractility in the organ bath containing 10

mL Kreb's Hanselheit solution. The contractility was recorded by transducers and connected to PowerLab apparatus. After maximal contraction reached and steady state contractility, 0.0625% of *Thymus vulgaris* extract for the first concentration was added to the organ bath, then waited for the response for seconds. Then it was continued with higher concentration of *Thymus vulgaris* extract (TM 0.125%, TM 0.25%, TM 0.5% and TM 1%). The lower concentration of *Thymus vulgaris* (0.0625%) decreased the contraction of smooth muscle cell in intact aorta; whereas, the higher concentration of *Thymus vulgaris* increased the contraction of aorta in intact aorta. The overall differences in smooth muscle relaxation of guinea pig aortic ring in each doses of TM in intact aorta are depicted in Figure 1 as follows.

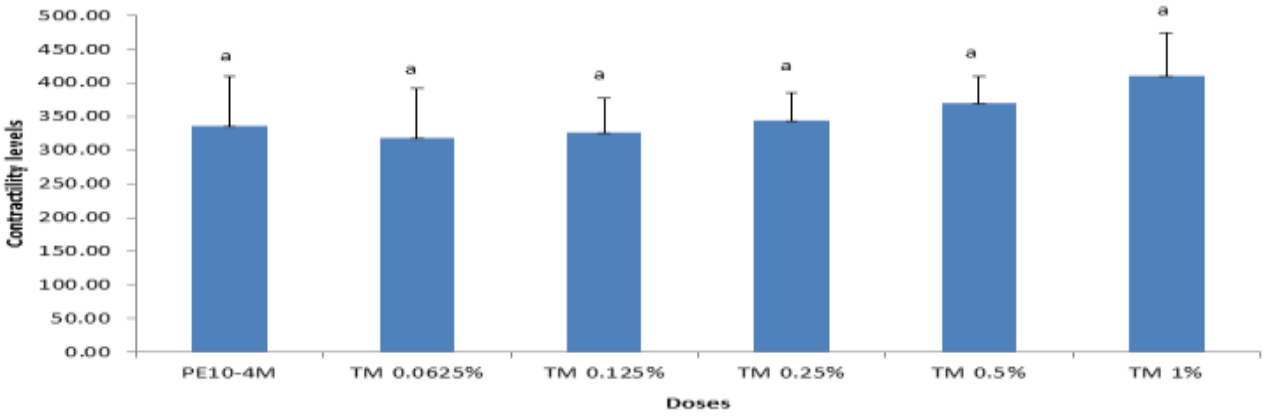

Figure 1: Smooth muscle contractility of guinea pig intact aorta ring

Based on the results of the correlation test, there was a correlation between the dose of *Thymus vulgaris* (TM) extract in intact aorta with contractility which correlation coefficient equal to 0.587 with a significance value equal to 0.021 ($p < 0.05$ This means that higher concentration doses of *Thymus vulgaris* extract will be followed by increasing contraction of smooth muscle in the intact aorta, and vise versa. And the regression test result, the coefficient of determination (R square) shows a value of 0.345. This means that 34.5% of the diversity of intact aorta is affected by *Thymus vulgaris* dose. While the remaining 65.5% is determined by other factors outside the variables studied.

The Effect of Different Concentrations of Thymus Vulgaris Extract on the Contractility of Denuded Aorta Guinea Pig

This study was done in order to find out the response of denuded aorta contractility, after the administration of different concentrations doses of *Thymus vulgaris* extract (0.0625%, 0.125%, 0.25%, 0.5% and 1%). The PE 10^{-4} M it was used to stimulated the contractility of denuded aorta in the organ bath. After the maximal contraction was reached and did not increased anymore, 0.0625 % of *Thymus Vulgaris* extract 0.1 ml for the first concentration was added to the organ bath, then the response was waited before recorded by powerlab computer. Then it was continued with the other concentrations (TM 0.125%, TM 0.25%, TM 0.5% and TM 1%) on the same way as intact aorta. The study showed that the lower concentrations of *Thymus vulgaris* causing decreased the contraction of smooth muscle cell in denuded aorta was doses 0.0625% and 0.125% ; whereas, the highest concentration of *Thymus vulgaris* causing increased the contraction on smooth muscle cell in denuded aorta was dose 1% . Added of *Thymus vulgaris* extract at concentration 0.0625% and 0.125% showed the best relaxation response on denuded aorta, and the administration of *Thymus vulgaris* extract at dose 1% increased the contractility after PE administration, which means it cannot give relaxation effect on denuded aorta.

In addition, giving *Thymus vulgaris* extract at concentration 0.25% and 0.5% can only increased the contractility level in intact aorta slightly, but still below the PE contractility which reflect that the relaxation response of smooth muscle cell in denuded aorta was not as good as the lower doses. The overall differences in smooth muscle relaxation of guinea pig aortic ring in each doses of TM in denuded aorta are depicted in Figure 2 as follows.

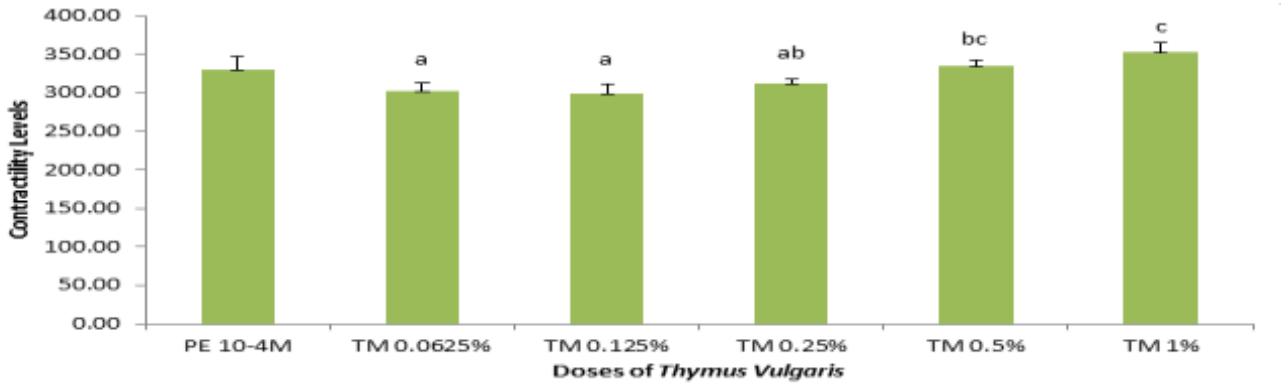

Figure 2: Smooth muscle relaxation of denuded aorta ring in each TM administration

Based on the results of the correlation test above shows that between the concentration of *Thymus vulgaris* (TM) extractand contractility of denuded aorta has a correlation coefficient equal to 0.903 with a significance value equal to 0.000 ($p < 0.05$), so it can be concluded that there was a significant relationship between dose with *Thymus vulgaris* (TM) extract indenuded aorta. The correlation coefficient was positive, meaning that the higher concentration doses of *Thymus vulgaris* extract followed by increased the contraction of smooth muscle in denuded aorta..

Comparison between Effect of Thymus Vulgaris Extract in Intact and Denuded Aorta

The T-test analysis was used to compared the effect of *Thymus vulgaris* extract on intact and denuded aorta of guinea pig. The average of Intact Aorta in the group PE 10^{-4} equal to 336.67, while the average of denuded aorta in the group PE 10^{-4} equal to 330.53, so that it can be concluded that there was no significant difference between the average of intact aorta and denuded aorta in the group PE 10^{-4} M. Overall its showed that *Thymus vulgaris* extract could be decreased contractility better in denuded aorta.

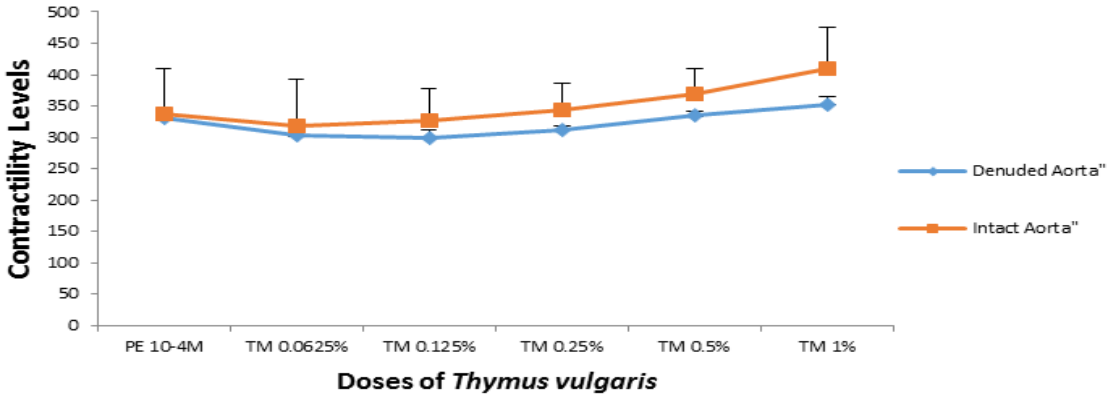

Figure 3: Comparison effect of *Thymus vulgaris* between intact and denuded aorta

Treatment of Thymus vulgaris *extract on the contractility of intact and denuded aorta of guinea pig*

Contraction of smooth muscle can be evoked by PE. The possiblity that PE could act directly to stimulate the endothelial cells has been noted by preliminary study [11]. in both endothelium intact and denoded aorta. PE bound with a1 adrenoreceptors on the membranes of vascular smooth muscle, PE mobilized intracelluler Ca^{2+} causing contraction of action and myosin filaments. The shortening of muslce cells decreases the diameter of arteriole, causing an increase in resistance to blood flow through vessel. PE was administered to the organ bath containing isolated aorta which began with the concentration of 10^{-7} M to10^{-2} M. The concentration effect of PE administration towards isolated Aorta concentration choose by average of value of 10^{-4} M because in this concentration gain the 70-80% effect. The drug applied in this research was acetylcholine 10^{-5} M with volume 0.1 ml.

It was administered on the pre-contracted guinea pig's aorta which had been stimulated

by PE 10^{-4} M. The result from introduction study was aorta relaxation occurred after the administration of acetylcholine 10^{-5} M. It showed that endothelium was intact because acethylcholine has its receptor in endothelium cells. The intact aorta (endothelium intact aorta of guinea pig) was used in this study. It is because endothelium is very important in order to release endotelium derived vasorelaxation substances such as NO and PGI2 [12]. To know the intact of endothelium, acetylcholine must be given towards pre-contracted aorta by PE. Acetylcholine would cause vasodilatation and lower blood pressure.

Although no innervations of vasculature by the parasympathetic system existed, there were cholinergic receptors on blood vessels responded by vasodilatation. The vasodilatation is due to an acetylcholine induced rise in intracelluler Ca2+, caused by the phosphatidylinositol system which results in the formation of nitric oxide (NO) from arginine in endothelia cells [13]. When the contraction decreased after the acetylcholine administration, it showed that endothelium was intact (aorta with endothelium). On the contrary when the contraction increased after the acetylcholine administration, it showed that endothelium was denuded (aorta without endothelium).

Effect of Thymus Vulgaris Extract on Intact Aorta of Guinea Pig

The data analysis of aorta contractility after adminitering 5 concentrations of *Thymus vulgaris* extract showed that 0.0625%, 0.125%, 0.25%, 0.5% and 1% were able to effects on intact and denuded aorta. From 3 repetitions of the intervention, the mean of aorta contraction in average for each contraction was obtained.

Supplementation of *Thymus vulgaris* could decreased and increased the aorta contractility both intact and denuded aorta in concentration dependent manner significantly ($P > 0.05$). This effect was caused by active compound in *Thymus vulgaris* extract. This result showed that increased doses of *Thymus vulgaris* extract concentrations could cause more contraction effect of guinea pig's aorta treated with and without endothelium. It meant there was a relationship between contractions and responses of aorta contractility. Furthermore, the data analysis of aorta contractility after administering five concentrations doses of *Thymus vulgaris* extract showed that *Thymus vulgaris* (TM) extract at a dose 1% can increase intact aorta to the average of the highest rather than the provision of TM extract at a lower doses. The concentration 0.0625% of TM extract showed decreasing contractility and increasing concentration gradually increased the aorta contractily until concentration 0.5% still under the maximal contractility of PE. And the highest concentration 1% increased the contractility higher that PE effect.

Thymus vulgaris appear effectively at 0.625% and 0.125% on the intact aorta analysis to inhibit activation of endothelial of smooth muscle via PE treatment. Activation of endothelial receptors and subsequent increase in Ca2+ levels causes K+ efflux from the cell. The smooth muscle cell responds to changes in the extracellular K+ levels and also releases K+ out of smooth muscle cell causing hyperpolarisation, thus the change in the membrane potential of the smooth muscle cell reduces intracellular Ca2+ levels, resulting in relaxation [12]. Thymol also decreased blood pressure through the endothelium independent relaxation [14].

Effect of Thymus vulgaris *Extract on Denuded Aorta of Guinea Pig*

The concentrations doses of *Thymus vulgaris* decreased aorta contractility at concentration 0.0625%, and gradually increased by 0.125%, 0.25%, 0.5% and 1% could increase denuded aorta endothelium contractility respectively. From 3 repetitions of the intervention, the mean of aorta contraction in average for each concentration was obtained. *Thymus vulgaris* has significantly affect intact aorta of guinea pig. This study confirms that *Thymus vulgaris* also effectively used as vasorelaxant in mural animal in low concentration.

In other study, thymol has been shown to exhibit vasorelaxant activities in the isolated rat aorta. In isolated rat aorta, endothelium-independent relaxation induced by thymol occurs via release of Ca^{2+} from the sarcoplasmic reticulum diminishing the sensitivity of contractile elements to Ca^{2+} and preventing the influx of Ca^{2+} across the membrane [15]. Based on the result, the effective doses for denuded aorta appear at 0.625%, 0.125% and 0.25%. These results represent the stable trend until 5 minutes.. But when the concentration of Thymus vulgaris increased by 0.5% and 1% resulted in high increasing the contractility.PE could

increase contractility because its binds with a1 adrenergic receptors. The activation of adrenegic receptor lead to activate G protein coupled receptor (G9) which activates phospholipase C, PLC which causes an increase in IP3 and calcium. This triggers further effects, primarily through the activation of an enzyme protein kinase C. This enzyme, as a kinase, functions by phosphorylation of other enzymes causing their activation, or by phosphorylation of certain channels leading to increase or decrease of electrolyte transfer in or out of the cell.

By releasing of the Ca+2 intracellular it causes contraction of actin and myosin filaments in the blood vessel [16]. *T. vulgaris* contains of several active compounds, one of them is thymol. At low dose thymol have effect on blocking voltage-activated sodium currents in smooth muscle and neuronal cells [14]. Administration thymol in smooth muscel cell suppressed calcium and potassium current in a concentration-dependent manner.

Thymol, at micro concentrations, reduced the activity of calcium dependent adenosine triphosphatase (Ca^{2+}-ATPase) and increased the permeability of Ca2+ in the sarcoplasmic membrane and so it will be increase the Ca2+ concentrations of neurons or of smooth muscle preparations. Thymol has agonistic effects for the adrenergic receptors (α1, α2, and ß) isolated from stomach and vena portae of guinea pigs on the circular smooth- muscle strips (SMAs).

Thymol (10−4 M) inhibits spontaneous contractile activity of the SMAs (100%) and diminishes the excitatory effect of acetylcholine chloride to 35%. *Thymus vulgaris* bound with alpha receptor which led to activate Gq protein and eventually led to Ca^{2+} liberation intracellular and contraction. This activated myosin light chain kinase and caused phosphorilation of myosin light chain. Therefore, the contraction of smooth muscle of isolated aorta occurred [17]. Besides, that medicinal plant might play a role to activate Ca^{2+} channel of smooth muscle of isolated aorta. Then the calcium intraceluler would increase and the formation of calcium calmodulin occurred.

Therefore the contraction of smooth muscle of isolated aorta occurred. Various doses of *Thymus vulgaris* led to more contractions of smooth muscle of aorta, unless the contraction was in absence of endothelial than that of endothelium [17]. Acting on endothelial cell induced NO and PG12 was released even though the vasodilation effect of each dose did not give a significant effect. Meanwhile various doses of *Thymus vulgaris* caused more contractions of smooth muscle of aorta in absence of endothelial than that of endothelium. It meant some them relaxed it. The relaxation of various Thymus vulgaris doses came from the activation of NOS leading to NO (nitric oxide) diffused to the adjancent vascular smooth muslce cell and activated guanyl cyclise enzyme pathways to for, cGMP [14,17]. The increase of cGMP would cause Ca^{2+} channel blockade.

Comparison Effect of Thymus Vulgaris *Extract on intact and Denuded Aorta*

Comparing result of *Thymus vulgaris* on intact and denuded aorta from range 290 until 326 which appear significant for intact and denuded aorta at doses 0.625% and 0.125% for intact and at doses 0.625%, 0.125%, and 0.25% for denuded aorta [18]. *T. vulgaris* have the antihypertensive effect as well as protective effects against hypertension induced aorta damage in hypertensive rats.Antihypertensive effect of *T.vulgaris* impact on the smooth muscle relaxant and vasorelaxant properties rather than in endothelial cells. Thymol significantly enhanced tonus resting both on endhotelium intact and denuded rat aortic ring [15]. Another mechanism by *T. vulgaris* induce vasorelaxant mediated through carvacrol pathway [19].

Vasodilatory effect of carvacrol occur through TRPV3 channels (transreceptor potential cation channel-3) present in the endothelium. Increases in endothelial intracellular Ca2+, through activation of TRPV3 channels activate intermediate-conductance (IKca) basolateral potassium channels and small conductance calcium (SKca) activated potassium channels, hyperpolarizing the plasma membrane of endothelial cells and underlying smooth muscle. Inwardly rectifying potassium channels (KIR), specific subset of potassium selective ion channels, in smooth muscle cells amplify this initial hyperpolarization, ultimately resulting in vasodilation.

These findings suggest that activation of TRPV3 channels in the endothelium may improve vascular function by promoting

arterial relaxation. This finding can results a novel mechanistic for cardioprotective benefits of a diet includes in amounts of *Thymus vulgaris* [19]. However *T.vulgaris* might act on the endothelial. It was because when *T.vulgaris* was given to the aorta, the contraction of aorta smooth muscle increased. The increased contractility due to active constituents bound with α1 receptors and eventually leaded to contractility although with endothelia cells the contractility response was not as much as without endothelial. Thymol is main compound of many meterpenes substances that contain in *T.vulgaris*.

Thymol has blocking effect on voltage activated sodium both in experimental cell models as well as animal and human origin research. In rat skeletal muscle fibers isolated enzymatically, thymol (30-600 ug) treatment suppressed both calcium (Ca^{2+}) and potassium (K^{+}) currents in a concentration dependent manner half-maximal effect (EC50) values of 193 ± 26 and 93 ± 11 mM and Hill coefficients of 2.52 ± 0.29 and 1.51 ±0.18 respectively [20]. Active constituents of these active constituents are responsible for relaxation effect. Furthermore, they may act on other receptors such as B receptors on the heart [17].

Concludes that *T.vulgaris* methanolic extracts can decrease the aorta contractility in guinea pig. Understanding the effect of thymus vulgaris can be very general, because there are many active compounds when it analyse further. But to consider the standard value of extract treatment on endothelial tissue other study mention fragmented sarcoplasmic reticulum as homeostasis calcium by which dominant of concentrations. Fragmented sarcoplasmic reticulum or FSR is observed to determine the balance between the rapid influx and efflux of calcium [21]. Thymol depresses the efflux of calcium uncoupled with Ca^{2+} ATpase activity that amount of calcium is increased in FSR in spite of partial deactivation of Ca^{2+} atp-ase. In this study, the concentration of calcium in FSR is determined by the balance between influx and efflux if calcium. Ca^{2+} ATPase may be partially deactivated by thymol, resulting in a depression of uptake ability for calcium. When the concentration of calcium in FSR reaches a certain critical value, the state of the thymol perturbated membrane is suddenly changed from a phase where the calcium permeability is small to another phase with large calcium permeability. This lead to a marked decrease in the amount of accumulated calcium in a short time.

Thus, *T. vulgaris* in this study consider as the effective dose both intact and denuded aorta (P>0.05). However, in this research the aorta intact and denuded was used to prove the target of *T. vulgaris*. The response of methanolic extracts of *T. vulgaris*, the mechanisms were unknown. The contractility increased instead of relaxation. Therefore, the suggestion for the future research is to explore the active substance which is responsible for contraction and relaxation of the vessels both in vitro and in vivo experiments.

The effectiveness of potential substance from *T. vulgaris* as smooth muscle relaxants must be further examined by doing clinical testing in order to observe the side effect and the therapeutic effect towards human. As the conclusion, T. vulgaris methanolic axtract has biphasic effect pharmacologically. In low concentration its has relaxation effect, but in higher dose it's could increased orta contractility both in intact and denuded aorta. So, Its seems that the target of T. vulgaris is on the smooth muscle cells of vessels.

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