ANTI-ATHEROSCLEROTIC EFFECT AND LIVER TOXICITY OF ETHANOLIC EXTRACT OF *PHALERIA MACROCARPA (*SCHEFF.) BOERL FRUIT ON JAPANESE QUAIL

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Abstract

An anti-atherosclerotic effect and liver toxicity of an ethanolic extract of *Phaleria macrocarpa* (Scheff.) Boerl fruit on Japanese Quail has been evaluated. Atherosclerosis was induced by treated the Japanese quail with atherogenic cocktail (a mixture of 5% cholesterol, 30 % coconut oil, 0.1% propylthiourasil, 2% chicken bile juice and water until 100 ml),1ml/100g BW orally once a day everyday for two months. Fife groups of animal (5 quail each, ± 3 month, 80-100 grams of body weight) were used. Ethanolic extract of *Phaleria macrocarpa* fruit was given orally to the animals once a day half an hour before cocktail, everyday for two months at doses of 50, 100 and 200 mg/kg to three groups, while a group of negative control and a group of positive control were used as comparison. Cholesterol level, the thickness of the aorta and coronary arterial walls, percentage of the vascular lumen width, the endotelial and vascular smooth muscle cells damage, liver ratio and serum glutamate-pyruvate transaminase (SGPT) activity were measured. Results show that the cholesterol level of Japanese quail was tend to decrease ($p<0.1$) under the influence of *Phaleria macrocarpa* fruit extract. The thickness of aorta and coronary arterial walls and the percentage of their widths were significantly improved ($p<0.05$), the endotelial and vascular smooth muscle cells damage of coronary artery was significantly reduced ($p<0.05$) while of the aorta was not reduced significantly ($p>0,1$) as compared to those on positive control animals. On the other sides, the liver ratio and SGPT activity of treated animals were higher as compared to those in controls ($p<0.05$). These data indicated that the ethanolic extract of *Phaleria macrocarpa'*fruit is able to prevent aortic and coronary arterosclerosis induced by atherogenic cocktail, but is toxic to liver if it is used for a long time.

Keywords: anti-atherosclerosis, Japanese quails, *Phaleria macrocarpa* fruit ethanolic extract, atherogenic cocktail, liver toxicity, liver artio, SGPT.

Introduction

Atherosclerosis is the most common cause of ischemic heart disease, which can lead to unstable angina, myocardial infarction ('heart attack'), stroke, and sudden death. Studies indicate that plaque composition rather than the degree of stenosis or blood vessel narrowing is the key factor for predicting vulnerability to rupture or

thrombosis (Fayad and Fuster, 2001; Fuster et al., 1999). One of a common cause of atherosclerosis is hypercholesterolemia (Anitschkow, 1913; Song et al., 1998; and Yang et al., 1998). Atherosclerosis is a disease indicated by a formation of plague atheroma due to a modification of the tunica intima of the arterial blood vessel as a result of cholesterol sedimentation (Guyton, 2006). The plague will lead to an arterial degeneration and narrowing and even obstruction its lumen. (Glomset, 1976 ; Price and Lorrainem, 1995).

Phaleria macrocarpa (Scheff.) Boerl., is classified as followed (Harmanto, 2002 and Harmanto, 2003) :

Divisio	: Spermatophyta
Sub Divisio	: Angiospermae
Class	: Dicotyledonnae
Ordo	: Celastreales
Family	: Thymealecea
Genus	: Phaleria
Species	: *Phaleria macrocarpa* (Scheff.) Boerl.

In general, atherosclerosis can be adalah prevented by a low cholesterol diet hypolypidemic drugs, such Probucol. Probucol can lower blood cholesterol beside antioxidant effect The hypolypidemic effect could be augmented if Probucol is combined with other hypolypidemic agents and HMG CoA reductase inhibitor (Finckh et al., 1991). God Crown or *Phaleria macrocarpa* (Scheff.) Boerl. Has been used by Indonesian people for several diseases, such as hyperlypidemia, diabetes Melitus, hypertension, antioxidant, analgesic, anti-inflamation (Anonymous, 2004 and Hermanto, 2003). hyperuricemia (Laksmitawati, and Ratnasari,, 2006), and cytotoxic (Supriyano, 2006) It was also described that the fruit and the leaves of this plant contain flavonoid (Anonymous, 2004).

This objectives of this study was to evaluate the anti-atherosclerosis effect of God Crown fruit ethanolic extract on an atherogenic cocktail induced atherosclerotic Japanese quails.

Material and Methods

Anti-Atherosclerotic evaluation of God Crown Fruit Extract

An amount of 25 Japanese quails was grouped into 5 (5 quails each) and treated as negative control (water), extract treated groups at doses of 50 mg/Kg BB, 100 mg/Kg BB dan 200 mg/Kg BB orally besides atherogenic cocktail (30% of coconut oil, 5% of cholesterol, 2% bile juice, 0,1% propylthiourasil, araic gum q.s and and waterad 25ml) (Clair, 1998) respectively and as a positive control (trated with atherogenic cocktail plus water only). Cocktail (oral) and extract (or water) (i.p) were given once a day every day for 2 months and the food and drinking water were given ad-libitum. On the day 61, the animals were killed, the blood was collected for cholesterol and SGPT measurements, the heart and aorta were removed for atheroma measurements and the liver was collected for liver ratio measurement.

Atherosclerotic Lession Measurement

The aorta and the heart were prepared for histopathologic measurement using confentinal methods with hematoxylline and eosine as the dies. The arterial thickness, the percentage of arterial lumen width and and the level of arterial endothel and vascular smooth muscle cell damage (score 1 for smaller level damage,

score 2 for middle level damage and score 3 for the higher level damage) were masured microscopically (Bajpai, 1989 and Leeson et al., 2002).

Liver toxicity measurement.

Liver toxicity was measured as the liver ratio to the body weight ($RatioBeratOrgan = \frac{BeratOrganHewan}{BeratBadanHewan}$), and SGPT activity which was measures spectroscopically (Shimadzu 160-A), (Kiernan, 1990).

Data Analysis

The data of liver ratio, SGPT activity, aorta and coronary arterial thickness, the percentage of arterial lumen width and the level of arterial smooth muscle damage were analysed statistically using one way ANOVA followed by Duncan post Hock test. Significant was taken at $p<0.05$.

Results and Discussion

Table 1 showed that arterial thickness of positive control (atherosclerosis induced artery) was thinner as compared to the thickness of negative control artery (water treatment only) and the God Crown ethanolic extract increased the thickness of aorta significantly ($p<0.05$) similar with that on negative control, especially at the higher doses (100 and 200 mg/kg). There was nosignificant different ($p>0.1$) in coronary arterial thickness betreen all groups of Japanese quails.

Table 1. The Influence of God Crown extract to Arterial Thickness on Javanese Quail ($^{a, b}$ = p<0.05)

Treatments	Arterial Thickness (µm)	
	Aorta	Coronary
Kontrol (-) ± SD	32,50 ± 4,333^{b}	26,500 ± 6,552
Kontrol (+) ± SD	22,11 ± 3,103^{a}	31,667 ± 6,552
Dosis 50 mg/Kg BB ± SD	22,84 ± 3,062^{a}	36,900 ± 6,552
Dosis 100 mg/Kg BB ± SD	30,11 ± 1,416^{b}	26,625 ± 6,552
Dosis 200 mg/Kg BB ± SD	34,11 ± 1,441^{b}	26,833 ± 6,552

On the other hands, arterial lumen of aorta treated with atherogenic cocktail was wider as compared to that in control negative quails (p<0.05) and the God Crown extract treated quails beside atherogenic cocktail showed a amaller aorta lumen, especially at the higher dose (Table 2). The coronary arterial lumen width was not significantly affected by the treatment.

Table 2. The Influence of God Crown extract to Arterial Lumen Width on Javanese Quail ($^{a, b}$ and ^{c} = p<0.05)

Treatments	Arterial Lumen Width (%)	
	Aorta	Coronary
Kontrol (-) ± SD	15,77 ± 11,051^{a}	32,237 ± 6,380
Kontrol (+) ± SD	41,63 ± 8,137^{b,c}	28,853 ± 6,380
Dosis 50 mg/Kg BB ± SD	43,18 ± 0,000^{c}	19,455 ± 6,380
Dosis 100 mg/Kg BB ± SD	29,47 ± 2,139^{a,b}	31,727 ± 6,380
Dosis 200 mg/Kg BB ± SD	17,91 ± 14,100^{a}	32,830 ± 6,280

Aorta endothel and smooth muscle cells of control positive quails was seen to be damaged by treating with atherogenic cocktail with the level of damage of 3 (compare to 0.75 on negative control quail) even though this damage was not significant (p>0.1). On the other hands, in coronary artery, the level of the damage

was significant ($p<0.05$) which can be corrected by treated with God Crown fruit extract (Table 3).

Table 3. The Influence of God Crown extract to Arterial Endothel and Cel Damagel on Javanese Quail ($^{a, b}$ and ^{c} = $p<0.05$)

Treatments	Arterial Endothel and Cell Damage	
	Aorta	Coronary
Kontrol (-) ± SD	0,75 ± 1,500	0,00 ± 0,629[a]
Kontrol (+) ± SD	3,00 ± 0,000	3,00 ± 0,629[c]
Dosis 50 mg/Kg BB ± SD	3,00 ± 0,000	3,00 ± 0,629[c]
Dosis 100 mg/Kg BB ± SD	1,67 ± 1,528	2,25 ± 0,629[c]
Dosis 200 mg/Kg BB ± SD	1,67 ± 1,155	1,00 ± 0,629[b]

Blood cholesterol level of atherogenic induced quail was higher than that in normal quail even though not significant ($p>0.1$), and God Crown reduced this level more. SGPT activity of normal and atherogenic induced quail were not significantly different ($p>0.1$) but this parameter was higher in God Crown + atherogenic cocktail treated quail ($p<0.05$). Further more, the liver ratio of all group of quails was not significantly different ($p>0.1$)(Table 4).

Table 4. The Influence of God Crown extract to Plasma Cholesterol, SGPT and Liver Ratio on Javanese Quail ($^{a, b}$ and ^{c} = $p<0.05$)

Treatments	Plasma Cholesterol, SGPT and Liver Ratio		
	Cholesterol (mg/dL)	SGPT U/L	Liver Ratio (x 10^{-2})
Kontrol (-) ± SD	210,58 ± 40,75	13,94 ± 4,390[a]	1,17 ± 0,1155
Kontrol (+) ± SD	274,51 ± 40,75	10,38 ± 4,480[a]	1,07 ± 0,1155
Dosis 50 mg/Kg BB ± SD	231,84 ± 40,75	18,39 ± 9,803[b]	1,17 ± 0,2309
Dosis 100 mg/Kg BB ± SD	236,13 ± 40,75	22,84 ± 4,390[b]	1,30 ± 0,1000
Dosis 200 mg/Kg BB ± SD	191,27 ± 40,75	36,79 ± 7,249[c]	0,97 ± 0,0577

God Crown (*Phaleria macrocarpa* (Scheff.) Boerl) fruit has been used for quite long tome to treat hiperkolesterolemia as described by Kirtishanti et al., (2004), Harmanto (2003) and Harmanu (2006).

Japanese quail (C*oturnix-coturnix japonica*) is very sensitive to kerentanan atherosclerosis and the proces of atherosclerosis lesion formation on this species is similar top that developed on human being (Clair, 1998). According Vogel (2002), the development of atherosclerosis on quail occure 2-3 moths after atherogenic cocktail. But Clair (1998) approved the the condition of atherosclerosis can be occured after 2 moths treatment wirh atherogenic cocktai, as seen in this study.

Cholesterol was used to induced hypercholesterolemia, by that mean an atherogenesis will be developed. Coconut oil very usefull both as a solven and to accenuate blood cholesterol and also trigyseride (Murray et al, 1997). PTU will cause a decrease of tyroid hormone (hypotyroidism) which accelerate hipercholesterolemia (Murray et al., 1997 and Guyton, 2006). In a normal condition, tyroidal hormone act to increase fat metabolism by increasing LDL receptor formation. So that, the reduction of its level will increase plasma lipoprotein level, especially one that contain cholesterol. The purpose to use bile juice in this study was increase fat metabolism in the gastrointestinal tract which was given in large amount to the quails (Clair, 1998). With all of these cobination, it was expected that the increase of cholesterol level be exist.

Several parameters were measured in this study, i.e. the thickness of arterial wall, the percentage of arterial lumen, the degree of endothel and smooth muscle cell damage, the ratio of liver to the body weight, the activity of SGPT and blood

cholesterol level. Remodelling of blood vessel due to atherosclerosis indicated commonly by the change of arterial wall thickness, lumen width and the degree of vascular smooth cell damage as seen in this study (Clarkson et al., 1994). To observe the adverse effect of long term administration of God Crown fruit extract to the liver, the liver ratio and plasma SGPT level were measured. As added data, plasma ckolesterol of the quails was measured. .

Two kind of artery were evaluated in this study, i. e. Aorta and coronary arteries. According to Guyton (2006), the large and middle size arteries are sensitive to atherosclerosis due to higher blood pressure (Di fiore, 1991; Ganong, 1995 and Leeson, 2002). Development athersclerosis on the coronary artery for a long time will lead to coronary arterial disease (Ivan, 1997).

The aortal wall or quail treated with atherogenic cocktail was thinner as compared to those in normal quail, while its lumen was larger. According to Hassan et al. (2002), An increase of plasma cholesterol may lead to smooth muscle cell apoptosis which cause linning of tunica media, decrease of arterial wall followed by its weakness. As the compensation, the diameter of the artery becomes wider (vasodilatation). Treating the quail with God Crown Extract prevent arterial damage induced by atherogenic cocktail according to doses. These effect may due to the blood cholesterol lowering effect of this extract as also seen in this study. In contrary of that in the aorta, the thickness of coronary artery under atherogenic cocktail was higher as compared to that in normal while the lumen wdth was smaller. God crown extract was able to return these two parameter to normal, eventhough not significant. Endothelial and vascular smooth muscle cells damage induced by

atherogebic cocktail was also tent to be prevented by this extract, in which, in coronary artery was significant ($p<0.05$). From this histopathological study, there were fat cell accumulation, cell proliveration and endothelial damage in the arterial (aorta) wall of the quail treated with atherogenic cocktail. These are as the preliminary change of the arterial wall towards atherosclerosis plague formation (Guyton, 2006). On the other sides, the complete atherosclerosis plague formation in the form of arterial wall calcification, lost of arterial wall elasticity and a arterial lumen narrowing were not seen in this study.

An interesting fenomena found in this study is that the arterial respons to atherogenic cocktail in aorta was contradictory as compared to that in coronary artery. When the aorta become dilated, the coronary artery was constricted. According to Guyton (1997), Price, S.A. and Lorrainem (1995) and Robins and Kumar (1995), atherosclerosis indicated with atheroma plague formation in the arterial wall, narrowing its lumen and endothelial cell and proliferation of arterial smooth muscle cell , as seen in tis study. Unfortunately, atheroma plague could not be measured due to technical problem, in which the plague may had been washed out or dissolved in alcohol used in the histological preparation. As the result, atherosclerosis was seen as a thickening of arterial (coronary) wall doe to amooth muscle cell proliferation which lead to arterial narrowing.

. As most other drug metabolism, God Crown extact also metabolized in the liver. This would increase the work capacity of the liver which lead to increase liver ratio (hypertrophy) and SGPT activity (Guyton, 2006).as seen in this study.

It can be concluded that the God Crown extract could prevent atherosclerosis induced by atherogenic cocktail on the Japanese quail, but long term used could could decrease liver function and increase liver ratio ($p<0.05$).

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