

Hypolipidemic and Antioxidant Effects of Black Tea Extract and Quercetin in Atherosclerotic Rats

Wahyu Widowati, Hana Ratnawati, Tjandrawati Mozefis, Dwiwati Pujimulyani, Yelliantty Yelliantty

Abstract—Background: Atherosclerosis is the main cause of cardiovascular disease (CVD) with complex and multifactorial process including atherogenic lipoprotein, oxidized low density lipoprotein (LDL), endothelial dysfunction, plaque stability, vascular inflammation, thrombotic and fibrinolytic disorder, exercises and genetic factor. Epidemiological studies have shown tea consumption inversely associated with the development and progression of atherosclerosis. The research objectives: to elucidate hypolipidemic, antioxidant effects, as well as ability to improve coronary artery's histopathology of black tea extract (BTE) and quercetin in atherosclerotic rats. Methods: The antioxidant activity was determined by using Superoxide Dismutase activity (SOD) of serum and lipid peroxidation product (Malondialdehyde) of plasma and lipid profile including cholesterol total, LDL, triglyceride (TG), High Density Lipoprotein (HDL) of atherosclerotic rats. Inducing atherosclerotic, rats were given cholesterol and cholic acid in feed during ten weeks until rats indicated atherosclerotic symptom with narrowed artery and foamy cells in the artery's wall. After rats suffered atherosclerotic, the high cholesterol feed and cholic acid were stopped and rats were given BTE 450; 300; 150 mg/kg body weight (BW) daily, quercetin 15; 10; 5 mg/kg BW daily, compared to rats were given vitamin E 60 mg/kg/BW; simvastatin 2.7 mg/kg BW, probucol 30 mg/kg BW daily for 21 days (first treatment) and 42 days (second treatment), negative control (normal feed), positive control (atherosclerotic rats). Results: BTE and quercetin could lower cholesterol total, triglyceride, LDL MDA and increase HDL, SOD were comparable with simvastatin, probucol both for 21 days and 42 days treatment, as well to improve coronary arteries histopathology. Conclusions: BTE and quercetin have hypolipidemic and antioxidant effects, as well as improve coronary arteries histopathology in atherosclerotic rats.

Keywords—Black tea, quercetin, atherosclerosis, antioxidant, hypolipidemic, cardiovascular disease.

I. INTRODUCTION

ATHEROSCLEROTIC cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide [1]. Atherosclerosis is an inflammatory condition of the blood vessels [2], is a multifactorial pathological process where inflammation and oxidative processes are key components

from fatty streak formation to plaque rupture and thrombosis [3].

Hyperlipidemia, resulting from the abnormalities of lipid metabolism, is one of the major risk factors for the development of CVD. The elevated levels of plasma lipids such as fatty acids, cholesterol, phospholipids and triglycerides can lead to the development of atherosclerotic plaques [4]. Atherogenic dyslipidemic profile that consists of elevated triglycerides, an excess of small dense LDL, and low levels of HDL [5]. Cholesterol is an essential structural and functional component of cell membranes, higher levels of cholesterol decrease the fluidity of erythrocyte membranes [6]. Excess Reactive Oxygen Species (ROS) can lead to the secondary production of aldehydes such as malondialdehyde (MDA) and hydroxynonenal, through lipid peroxidation [2]. Oxidatively modified LDL initiates atherogenic processes including inflammation, platelet aggregation and smooth muscle cell proliferation [7]. Oxidized LDL has been identified as a main component in atherosclerotic lesions [2].

Epidemiological studies have shown an inverse correlation between diets rich in polyphenols and reduced risk of CVD [8]. Polyphenols constitute the most interesting group of *Camellia sinensis* components, and polyphenols, particularly flavonoids. Epidemiologic studies have reported a reduced risk of CVD in subjects with a high flavonoid intake through tea and other dietary sources [9], [10]. The potential protective effect of tea flavonoids has been attributed to antioxidant, antithrombogenic and antiinflammatory properties [10], [11]. Alterations in the *C sinensis* manufacturing process result in black, green, and oolong tea, which account for approximately 75%, 23%, and 2% of the global production, respectively [12], [13]. Tea leaves destined to become black tea are rolled and allowed to ferment (oxidize), resulting in relatively high concentrations of theaflavins and thearubigins and relatively low concentrations of catechins. Tea also contains small amounts of flavonols (kaempferol, quercetin and myricetin) in the form of glycosides [14].

Based on the epidemiological studies and bioactivities as well as component of black tea is potential to reduce atherosclerotic risk, it is important to elucidate hypolipidemic, antioxidant effects, as well as ability to improve coronary arteries histopathology of BTE and quercetin in atherosclerotic rats.

Wahyu Widowati (Corresponding author) and Hana Ratnawati are at Medical Research Center, Faculty of Medicine, Maranatha Christian University, Bandung, Indonesia (e-mail: wahyu_w60@yahoo.com).

Tjandrawati Mozefis is at Research Center for Chemistry, Indonesian Institute of Sciences, Bandung and 5Aretha Medika Utama Biomolecular and Biomedical Research Center, Bandung, Indonesia.

Dwiwati Pujimulyani is at Faculty of Food Technology, Mercu Buana University, Yogyakarta, Indonesia.

Yelliantty Yelliantty is at Aretha Medika Utama, Biomolecular and Biomedical Research Center, Bandung, Indonesia.

II. MATERIALS AND METHOD

A. Extract Preparation

Dried black tea leaves from Walini Tea Manufacturer (PTPN VIII, Bandung), tea plantation located in Cigaruni, Garut, West Java, Indonesia. One kilogram of dried black tea was extracted with distilled 70% ethanol by maceration extraction, filtered and evaporated using rotatory evaporator in 40°C. Our process resulted ethanol extract of black tea 119g (11.9%), The ethanol extract of black tea were stored at 4°C.

B. Animals and Treatment

Male Sprague-Dawley (SD) rats (6 weeks old, 140-170 g BW) were obtained from the National Agency of Drugs and Food Control (Jakarta, Indonesia). The rats were housed in plastic-bottom wire-upper cages and acclimated under laboratory conditions (25-27°C, humidity 60%, 12-h light/dark cycle) for 2 weeks to 165-190 g BW. Rats were kept in single system cages, each cage contains 1 rat. *In vivo* assay in rats were carried out at Pusat Antar Universitas (Animal Research Center, Center of Inter-university, Gadjah Mada University, Yogyakarta, Indonesia). The research has been approved by the Research Ethic Committee from Faculty of Medicine, Maranatha Christian University and Immanuel Hospital, Bandung, Indonesia.

After acclimation, the rats were fed basal diet (water content 12%, crude protein 15%, crude fat 3-7%, crude fiber 6%, Ca 0.9-1.1%, P 0.6-0.9%, 4400 kkal) and cholesterol diet for atherosclerotic inducer consist of basal diet supplemented 1.25% cholesterol (Sigma Aldrich) and 0.5% cholic acid (Sigma Aldrich). Rats were fed cholesterol diet for 2 months to reach atherosclerotic symptom. To check lipid profile, rats were measured cholesterol total, TG, HDL and LDL (Table I).

The profile lipid of rats indicated that the rats had hyperlipidemia. To know rats with cholesterol diet have atherosclerotic, coronary artery of rat samples were observed. Rats were fed cholesterol diet indicated hyperlipidemia and coronary artery narrowed and formed foamy cells. After rats positively atherosclerosis, cholesterol diet was stopped, and rats were fed basal diet for 42 days, rats were divided into 11 groups (n=5) for different treatment. The first group of atherosclerotic rats was positive control. The second group of rats was untreated (negative control). The third, fourth and fifth groups of rats were treated with BTE 450, 300, 150 mg/kg BW daily. The sixth, seventh and eighth groups of rats were treated with quercetin 15, 10, 5 mg/kg BW daily. The ninth, tenth and eleventh groups of rats were treated with vitamin E 60 mg/kg BW, simvastatin 2.7 mg/kg BW and probucol 30 mg/kg BW daily. The first observation was 21 days treatment by collecting of 1.5mL blood from the orbital vein in tubes. The experiment was terminated after 42-d treatment. Before rats were terminated, blood was collected from orbital vein, serum was separated for testing of total cholesterol, TG, HDL, and LDL, SOD level and plasma for testing of MDA level.

The rats were then anesthetized using ketamin 10% (50 mg/kg BW) and ilium-zylazil-20 (12 mg/kg BW) with perfusion method, or including coronary artery were collected and prepared for histopathology preparation.

C. Sample Preparation for Lipid Profile, Antioxidant, MDA Test

Blood 1.5mL from the orbital vein were collected in tube. The samples were centrifuged at 3000rpm for 10min and the serum was used for measuring the total cholesterol, LDL, HDL, triglyceride, SOD level. Blood 1ml were collected in EDTA and yielded plasma for MDA assay.

The total cholesterol was measured according to the manufacturer's instructions of kit from Cholesterol FS, GPO-PAP": enzymatic photometric method (DiaSys GmbH Germany), triglyceride from Triglyceride FS, "GPO" colorimetric enzymatic method (DiaSys GmbH Germany), HDL from HDL Precipitant (DiaSys GmbH Germany), "CHOD-PAP": photometric method and LDL from LDL Precipitant, "CHOD-PAP": photometric method (DiaSys GmbH Germany).

Serum SOD activity was determined by the commercial kit from Randox (Randox Laboratories) [15].

MDA activity an index of free radical generation/ lipid peroxidation, was determined as described by Okhawa et al. [16].

D. Histological Analysis

After mechanical testing, coronary artery was fixed by immersion in neutrally buffered 10% formalin, followed by dehydration and embedding in paraffin wax using standard procedures. Hematoxylin and eosin-stained sections of coronary artery were examined for signs of atherosclerosis.

E. Statistical Analysis

The data were analyzed using ANOVA followed by Tukey's HSD post hoc test to assess the statistical significance ($p < 0.05$) between treatment and control groups through all experiments. The data were expressed as the mean and standard deviation.

III. RESULTS AND DISCUSSION

Cholesterol 1.25% (w/w) and cholic acid 0.5% (w/w) were supplemented in food for 2 months significantly increased the cholesterol total, LDLI and TG and decreased the HDL compared to basal diet (Table I).

TABLE I
LIPID PROFILE OF RATS GIVEN CHOLESTEROL DIET FOR 2 MONTHS

Treatment period / High cholesterol diet	cholesterol	Lipid profile triglyceride	HDL	LDL
Day 0	89.96	58.61	119.09	17.40
1 month	187.20	101.85	52.09	32.81
2 month	198.41	104.41	48.72	56.69

After rats suffered atherosclerotic symptom (hyperlipidemic and narrowed artery and foamy cells in the artery's wall, can be seen at Fig. 1 (c)) rats were stopped consuming cholesterol diet and rats continuing fed various dose of black tea extract, quercetin, simvastatin, probucol for 21 days and 42 days. ANOVA showed that the treated groups were significantly different ($p < 0.01$) for all parameter including lipid profile (total cholesterol, LDL, TG, HDL), antioxidant activity (SOD), lipid peroxidation (MDA), to know the difference each other among treatment the data analyzed using Tukey's post hoc test (SPSS 20).

The hypocholesterol and antioxidant effects of BTE, quercetin in atherosclerotic rats can be seen at Table II for 21 days treatment and Table III for 42 days treatment.

Positive control for 21 days treatment (rats given cholesterol diet) exhibited higher cholesterol, LDL, TG, MDA and lower HDL, SOD compared to negative control. Black tea extract, quercetin, simvastatin, vitamin E, probucol for 21 days treatment in atherosclerotic rats could lower cholesterol,

LDL, TG and MDA level as well as increase HDL and SOD. BTE 450mg/kg BW daily was most active among BTE, quercetin 15mg/kg BW was the most active among all treatment to lower cholesterol. Simvastatin, vitamin E, probucol were similar activity to lower cholesterol level. BTE 300mg/kg BW, quercetin 10mg/kg BW were highest activity to lower TG. Simvastatin, vitamin E, probucol were similar activity to lower triglyceride level. Quercetin 15mg/kg was the most active to lower LDL level. BTE 450; 300mg/kg BW, vitamin E and probucol had similar activity to lower LDL level. Black tea extract 450mg/kg BW, quercetin 15mg/kg BW were similar and the most active to increase HDL level. Vitamin E and probucol were similar activity to increase HDL level. Vitamin E was the highest activity to increase SOD level. BTE 450mg/kg BW, quercetin 15mg/kg BW, simvastatin, probucol were similar activity to increase SOD level. Quercetin 5mg/kg BW was the most active to lower MDA level.

TABLE II
EFFECT BTE, QUERCETIN TOWARDS LIPID PROFILE, ANTIOXIDANT STATUS, LIPID PEROXIDATION IN ATHEROSCLEROTIC RATS FOR 21 DAYS TREATMENT
(DATA WERE EXPRESSED AS MEANS, STANDARD DEVIATION, TUKEY'S HSD POST HOC TEST)

Sampel	Cholesterol total (mg/dL)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dL)	SOD (U/g Hb)	MDA (mmol/l)
Postive Control	219.45±4.28	117.16±5.28	48.62±3.98	64.84±4.84	411.86±17.57	7.96±0.48
Negative Control	97.07±4.85	75.37±4.63	106.49±2.67	23.95±3.14	703.39±41.95	1.18±0.19
BTE	169.01±8.39	102.39±3.75	65.59±2.03	53.25±3.99	618.65±16.94	6.01±0.62
450 mg/kg	c	bc	d	bc	c	c
BTE	192.73±7.93	99.40±4.43	58.14±4.30	54.14±3.93	506.78±44.20	7.28±0.26
300 mg/kg	ef	b	bc	bc	b	def
BTE	206.01±6.04	112.09±4.03	55.56±2.16	59.75±3.70	401.70±15.39	7.59±0.14
150 mg/kg	fg	cd	bc	cd	a	ef
Quercetin	154.15±3.16	99.70±4.37	66.24±3.02	51.59±5.48	611.86±16.30	4.91±0.30
15 mg/kg BW	b	b	d	b	c	b
Quercetin	178.18±5.93	106.12±4.07	60.06±3.42	58.60±3.49	506.78±26.39	6.76±0.47
10 mg/kg BW	cd	bc	bcd	bcd	b	cd
Quercetin	206.01±5.90	110.30±5.26	54.41±2.56	57.58±3.38	410.17±36.25	7.64±0.12
5 mg/kg BW	fg	cd	ab	bcd	a	ef
Vitamin E	186.25±5.90	104.48±5.33	59.68±2.10	55.16±3.86	642.37±20.23	7.28±0.43
60 mg/kg BW	de	bc	bcd	bc	cd	def
Simvastatin	190.20±8.95	103.28±4.31	61.99±3.77	57.71±1.66	630.51±25.14	7.14±.035
2.7 mg/kg BW	de	bc	cd	bcd	c	de
Probucol	184.19±9.03	104.78±4.74	60.32±3.71	52.87±2.63	628.81±31.37	7.02±0.4 0
30 mg/kg BW	de	bc	bcd	bc	c	de

Data are presented as mean ± standard deviation. Different letters in the same column (among treatments) are significant at $P < 0.05$ (Tukey's HSD post hoc test).

Postive control for 42 days treatment (rats fed cholesterol diet) exhibited higher cholesterol, LDL, TG, MDA and lower HDL, SOD compared to negative control. BTE, quercetin, simvastatin, vitamin E, probucol for 42 days treatment in atherosclerotic rats could lower cholesterol, LDL, TG and MDA level as well as increase HDL and SOD. Quercetin 15

mg/kg BW was the most active among all treatment to lower cholesterol. Simvastatin, vitamin E, probucol were similar activity to lower cholesterol level. BTE 300mg/kg BW was highest activity to lower triglyceride. Simvastatin, vitamin E, probucol were similar activity to lower triglyceride level. BTE 300, 150mg/kg BW and quercetin 5mg/kg were the most

active to lower LDL level. Vitamin E, simvastatin and probucol were similar activity to lower LDL level. BTE 450 mg/kg BW, quercetin 15mg/kg BW were similar and the most active to increase HDL level. Vitamin E and probucol were similar activity to increase HDL level. BTE 450mg/kg BW,

quercetin 15mg/kg BW, simvastatin, vitamin E and probucol were similar activity to increase SOD level. Vitamin E was the most active to lower MDA level.

The histopathology of coronary artery of all treatment can be seen at Fig. 1.

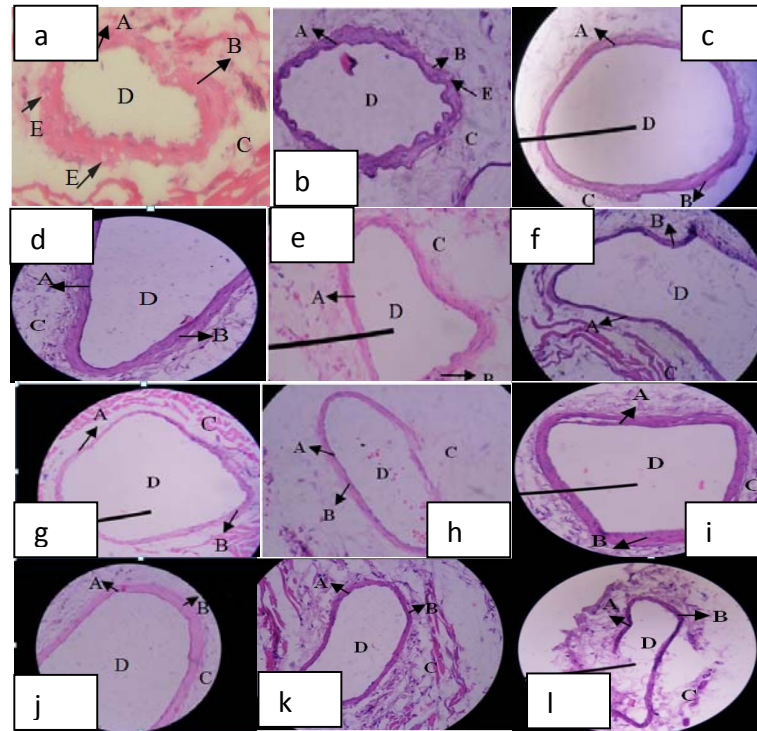


Fig. 1 Histopathology of coronary artery of treatment group (400x), (a) : positive control 0-d; (b) positive control 42-d; (c) negative control 42-d; (d) BTE 450mg; (e) BTE 300mg; (f) BTE 150mg; (g) Quercetin 15mg; (h) Quercetin 10mg; (i) Quercetin 5mg; (j) Vitamin E; (k) Simvastatin; (l) Probuco. A = intimal layer with endothelial lining; B = medial layer; C = adventitial layer; D = lumina; E = foam cell

The histologic features of coronary artery of positive control both 0-d and 42-d treatment showed that the intimal layer becomes thicker and the elastic membrane of the intimal layer fragmented and focally lost, narrowed artery's wall, formed foamy cells, the medial layer were thinner and the diameter of the luminalis smaller due to the atherosclerotic plaques.

The histopathologic of the coronary artery of the BTE (450; 300; 150mg/kg BW) and quercetin (15; 10; 5mg/kg BW), vitamin E, simvastatin, probucol showed almost normal artery, without plaque and foam cell A large lumen of the vascular with the intima showed a lining layer of endothelial cells, smooth muscle cells in the medial layer, while the adventitial layer composed of collagen, elastic and fibrous tissue.

The data in Table I showed that cholesterol diet (1.25% w/w) and cholic acid (0.5% w/w) could lead hyperlipidemia, consistent with previous study that Sprague Dawley rats with high levels of cholesterol (~1% w/w) and cholic acid (0.25% - 0.5% w/w) are capable to elevate triglyceride and LDL [17], likely by reducing bile acid production [18]. Foods high in dietary saturated fat (SF) and cholesterol have been linked to

elevations in circulating cholesterol levels in particular LDL [19]. Rapid lesion development has been achieved with high levels of dietary cholesterol (2% to 4% by weight), which has resulted in exceedingly high total plasma cholesterol levels and in lesions morphologically dissimilar to those seen in humans [20].

Positive control group which rats suffered hyperlipidemia as well as had oxidative stress with high MDA level, this data was validated with previous research that oxidative modification of LDL plays a crucial role in the development of atherosclerosis. Rats with combined hyperlipidemia have increased levels of circulating ox-LDL compared to negative control [21].

TABLE III

EFFECT BTE, QUERCETIN TOWARDS LIPID PROFILE, ANTIOXIDANT STATUS, LIPID PEROXIDATION IN ATHEROSCLEROTIC RATS FOR 42 DAYS TREATMENT
(DATA WERE EXPRESSED AS MEANS, STANDARD DEVIATION, TUKEY'S HSD POST HOC TEST)

Sampel	Cholesterol total (mg/dL)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dL)	SOD (U/g Hb)	MDA (mmol/l)
Postive Control	224.19±5.38 g	118.81±5.37 f	47.20±4.03 a	67.39±5.65 f	394.92±17.57 a	4.74±0.29 f
Negative Control	100.55±4.31 a	77.01±4.79 a	107.91±3.29 e	25.61±3.07 a	689.83±36.74 c	0.79±0.12 a
BTE	135.49±8.81 bc	90.30±4.02 bc	99.16±2.38 d	45.61±2.05 c	649.15±19.51 c	1.43±0.10 c
450 mg/kg BTE	160.32±8.41 ef	87.46±3.00 b	92.22±4.47 bc	38.47±4.52 b	540.68±35.15 b	2.55±1.60 e
300 mg/kg BTE	171.07±5.08 f	98.21±3.45 cd	89.00±2.11 b	35.03±2.26 b	444.07±19.51 a	2.73±0.10 e
150 mg/kg Quercetin	121.74±2.96 b	99.70±4.37 cd	99.56±3.47 d	44.84±3.33 c	647.46±17.57 c	1.17±0.17 bc
15 mg/kg BW Quercetin	145.77±5.93 cd	106.12±4.07 de	93.76±3.323 bcd	40.00±3.04 bc	535.59±23.52 b	2.23±0.26 d
10 mg/kg BW Quercetin	171.70±5.37 f	110.30±5.26 ef	88.49±2.97 b	34.39±3.25 b	444.07±40.91 a	2.74±0.08 e
5 mg/kg BW Vitamin E	152.41±6.53 de	90.60±5.32 bc	93.12±2.38 bcd	54.78±4.32 d	679.66±16.30 c	0.77±0.10 a
60 mg/kg BW Simvastatin	156.20±8.49 de	90.60±4.31 bc	95.69±3.56 cd	57.96±2.11 d	677.97±13.40 c	1.07±0.20 abc
2.7 mg/kg BW Probucol	150.36±8.92 de	92.24±4.17 bc	93.50±3.66 bcd	53.12±2.45 d	664.41±17.57 c	1.02±0.22 ab
30 mg/kg BW						

Data are presented as mean ± standard deviation. Different letters in the same column (among treatments) are significant at P < 0.05 (Tukey's HSD post hoc test).

Based on the data in Tables II and III, BTE could lower cholesterol, TG, LDL and increase HDL, this data were validated with previous finds that black tea reduces total and LDL cholesterol in mildly hypercholesterolemic adults [22]. Black tea is a major source of flavonoids, have antioxidant effects that may help to retard atherosclerosis [23]. A possible mechanism for the cholesterol-lowering effect of tea may be that tea limits cholesterol absorption in the intestine [24]. Green and black teas were equally effective in inhibiting atherosclerosis with the lower dose decreasing it 26-46% and the high dose decreasing it 48-63%, improvement in plasma LDL, LDL/HDL ratio, TG, lipid peroxides, lower density lipoprotein lipid peroxides, and fibrinogen [25]. Hypertriglyceridemia was normalized by green and black tea drink during 18 day, 25 day, respectively [26]. Green tea at 0.5 and 1.0% can decrease plasma and liver TG. The lipid-lowering effect of green tea is mediated partly by its inhibition of hepatic lipogenesis involving SREBP-1c and its responsive genes without affecting lipoprotein assembly [27]. Tea catechins decreased plasma total cholesterol, cholesterol ester, and atherogenic index. The results demonstrate that tea catechins exert a hypocholesterolemic effect in cholesterol-fed rats [28]. In animals fed diets high in fat and cholesterol black tea and tea polyphenols prevented elevations in serum and liver lipids, decreased serum total cholesterol or atherogenic index, and increased fecal excretion of total lipids and cholesterol [29], [30]. Increased consumption of tea is associated with decreased serum levels of TC and LDL [31]. Tea catechins can reduce plasma cholesterol levels and the rate of cholesterol absorption [32], [33]. Lipid metabolism studies in animals, tissues, and cells have found that tea extract and catechins reduce triacylglycerol and total

cholesterol concentrations [34], [35], inhibit hepatic and body fat accumulation [35]. A large Chinese study found that one capsule of a concentrated black tea extract (equivalent to 7 cups of black tea/day) reduces 16% LDL in hypercholesterolemic subjects on a low fat diet [36]. In an American population with mildly elevated cholesterol, consumption of 5 cups of black tea reduced significantly cholesterol, LDL, and lipoprotein (a) [37]. US epidemiology study reported that consumption of 2 or more cups of tea/day cut the risk of heart attack death in half [8]. Acute consumption of black tea increases antioxidant activity [24], chronic consumption of tea reduced the susceptibility of LDL to oxidation *ex vivo*. Oxidative modification of LDL plays an important role in the development of atherosclerosis [38]. Tea intake is associated with a reduction of CVD risk; two major factors contributing to the pathophysiology of atherosclerosis are hyperlipidemia and inflammation [39].

The data in Tables II and III exhibited that quercetin could improve cholesterol, TG, LDL, and increase HDL in hyperlipidemic rats. This results were consistent with previous research that quercetin is one of polyphenols group may protect against atherosclerosis by exerting hypocholesterolemic effects [40]. Phenolic compounds extracted from green and black olives such as quercetin exhibited an antihyperlipidemic action, reduced the lipid peroxidation process and enhanced the antioxidant defense system [41]. Combination consisting curcumin with piperine and quercetin (CPQ) were given in hyperlipidemic rats showed that CPQ reduced significantly TG 61.26%; LDL 66.69%, cholesterol 66.69% and increased HDL 24.45% [42], quercetin improved dyslipidemia [43]. The hypolipidemic activity of epicatechins in hamster is most likely related to the

inhibitory action on absorption of dietary fat and cholesterol rather involves the inhibition of synthesis of cholesterol or fatty acid [34], [44].

The data in Tables II and III exhibited that black tea extract could increase SOD activity and lower MDA compared to positive control, this data were consistent with previous research that black tea extract were effective in increasing the total plasma radical-trapping antioxidant status [24], [25], because black tea extract has high antioxidant activity [45]. Previous studies have demonstrated that oxidation of human LDL is one of the risk factors in the development of atherosclerosis and that dietary antioxidants lower the incidence of coronary heart disease [46]. Green and black teas were equally effective in reducing early atherosclerosis in hamsters fed a cholesterol/saturated fat diet. The tea effect was multifactorial and was the result of hypolipidemic, antioxidant, and hypofibrinogenic [25]. Tea contains catechins which may reduce LDL oxidation, thiobarbituric acid reactive substances (TBARS) formation, cellular oxidation, and superoxide production [47], [48].

Based on the data in Tables II and III exhibited that quercetin could improve SOD activity and reduce MDA both for 21-d and 42-d treatment, this data was consistent with previous study that quercetin modulates the deleterious inflammatory effects in hypocholesterolemic rabbits, quercetin has beneficial effect in decreasing inflammation in atherosclerotic progression [49]. Therefore antioxidants are strongly acted as effective anti-atherosclerotic agents. A variety of quercetin metabolites are known to be present in the circulation when quercetin-rich diet is supplied into the body [50]. One of quercetin metabolites is quercetin-3-O- β -glucuronide (Q3GA) possesses a considerable antioxidant activity and is capable of inhibiting copper ion-induced LDL oxidation [51]. Quercetin and metabolites *in vivo* are capable of inhibiting inflammation pathway through vascular leukotriene B₄ (LTB₄) [40]. TBARS contents and the cholesteryl ester hydroperoxides (ChE-OOH) level in the aorta tissue were also suppressed by the administration of quercetin glucosides, indicating that quercetin metabolites exert an antioxidant activity in cholesterol-rich aorta [52]. Quercetin metabolites possess xanthine oxidase (XOD) inhibiting activity in hypercholesterolemia which increasing endothelial superoxide production via XOD [53]. Quercetin metabolites can prevent ROS-induced injury [50]. Quercetin is an effective inhibitor of xanthine oxidase and lipoxygenase, enzymes involved in processes inflammation atherosclerosis [44].

Specific dietary polyphenols, in particular quercetin and theaflavin as component of black tea [54], it may attenuate atherosclerosis in ApoE^{-/-} gene-knockout mice by alleviating inflammation, improving NO bioavailability, and inducing hemeoxygenase-1. Cardiovascular protection associated with diets rich in fruits, vegetables, and some beverages may in part be the result of flavonoids, such as quercetin [40]. Antioxidant polyphenol protection against atherosclerosis may involve their antioxidant properties. Quercetin and catechins in tea have been shown to inhibit atherosclerosis. Oxidative stress in

the vasculature was effectively attenuated by quercetin, as demonstrated by significant reduction of aortic F₂-isoprostanes and superoxide [40]. Quercetin chelates ROS induced by lipid peroxidation and metal ions, provides H⁺ ions to prevent lipid peroxidation in the cell membrane, and scavenges free radicals. Furthermore, quercetin converts ROS to energy and reduces metal concentrations to protect cell membranes [55].

Probucol could improve hypercholesterolemia and increase SOD as well as decrease MDA level, this data was consistent with previous research that probucol is a potent LDL-lowering agent with powerful antioxidant property that effectively inhibits the oxidative modification of LDL [56].

Simvastatin could decrease LDL, TG cholesterol, MDA level as well as increase HDL, SOD level; this data was validated with previous research that statin as HMG-CoA reductase inhibitors are potent lipid-modifying agent reduces plasma LDL, decrease ROS generation. Statins block expression of protein subunits of p22^{phox} and gp91^{phox} which determine activity of NAD(P)H oxidases and expression of GTP-ase [NAD(P)H activator]. This leads to suppression of activity of pro-oxidant enzyme systems [NAD(P)H oxidase, xanthine oxidase, oxidase activity of endothelial NOS] and diminishment of production of most aggressive free radicals-superoxide anion and peroxynitrite. Hyperproduction of these radicals is associated with lowering of NO level and augmented NO destruction, the state of oxidative stress and endothelial dysfunction. Statins increase expression of enzymes with antioxidant properties (catalase and paroxonase), augment resistance of LDL to oxidation. Thus statins are considered as powerful antioxidants [56]-[58]. Simvastatin significantly reduced circulating ox-LDL in hyperlipidemia rats [21].

Vitamin E had hypolipidemic and antioxidant activity as well as reduces atherosclerotic lesion (Tables II and III, Fig. 1); this data was validated with previous finds that Vitamin E suppresses hypercholesterolemic atherosclerosis [59], [60]. Epidemiological studies indicate an inverse relationship between vitamin E intake and CVD, Increasing vitamin E intake is associated with a lower risk of coronary artery disease [61].

Fig. 1 showed that black tea extract could make wider lumen of the vascular with the intima showed a lining layer of endothelial cells, smooth muscle cells in the medial layer, while the adventitial layer composed of collagen, elastic and fibrous tissue, this data validated with previous research that catechins in black tea prevent vascular inflammation that plays a critical role in the progression of atherosclerotic lesions. The antiinflammatory activities of catechins may be due to their suppression of leukocyte adhesion to endothelium and subsequent transmigration through inhibition of transcriptional factor NF- κ B-mediated production of cytokines and adhesion molecules both in endothelial cells and inflammatory cells [62].

Fig. 1 exhibited that quercetin could improve coronary artery, this data validated with previous research that quercetin

has anti-inflammatory effects in the aorta may contribute to the attenuation of atherosclerosis [63], quercetin reduces the expression of human CRP and cardiovascular risk factors in mice [63].

Simvastatin, probucol and vitamin E could improve coronary artery, improve lumen of the vascular, this data was validated with previous data that statin, probucol and vitamin E could act hypolipidemic, antioxidant activity so could reduced ox-LDL and inflammatory as well as reduced atherosclerotic plaque.

IV. CONCLUSIONS

Black tea extract and quercetin have hypolipidemic and antioxidant activities as well as improve histopathology of coronary artery.

Vitamin E, simvastatin, probucol have hypolipidemic and antioxidant activities as well as improve histopathology of coronary artery.

ACKNOWLEDGMENT

We are grateful to Directorate General for Higher Education, Ministry of National Education of Republic Indonesia, for Research Grant of Hibah Kompetitif Penelitian Sesuai Prioritas Nasional (2009-2011) for financial support. We acknowledge gratefully to PAU (Pusat Antar Universitas) and Faculty of Veterinary, Gadjah Mada University, Yogyakarta for technical assistance and facilities support.

REFERENCES

- [1] Tavidou A, Manolopoulos VG. 2008. Novel molecules targeting dyslipidemia and atherosclerosis. *Curr Med Chem*; 15:792-802
- [2] Vasdev S, Gill V, singal PK. 2006. Beneficial effect of low ethanol intake on the cardiovascular system: possible biochemical mechanisms. *Vasc Health Risk Manage*; 2(3) 263-276.
- [3] Roy H, Bhardwaj S, Yla-Herttuala S. 2009. Molecular genetics of atherosclerosis. *Hum Genet* 125:467-491.
- [4] Jain K S, Kathiravan M K, Somani RS, Shishoo C J, Bioorg. 2007. *Med Chem*; 15:4674.
- [5] Chan JC, Cheung JC, Stehouwer CD, Emeis JJ, Tong PC, Ko GT, Yudkin JS. 2002. The central roles of obesity associated dyslipidaemia, endothelial activation and cytokines in the metabolic syndrome: an analysis by structural equation modeling. *Int J Obes Relat Metab Disord*; 26(7):994-1008
- [6] Duchnowicz P, Broncel B, Podsek A, Koter-Michalak M. 2012. Hypolipidemic and antioxidant effects of hydroxycinnamic acids, quercetin, and cyanidin 3-glucoside in hypercholesterolemic erythrocytes (in vitro study). *Eur J Nutr*; 51:435-443 DOI 10.1007/s00394-011-0227-y
- [7] Stocker R, Keane JF Jr. 2004. Role of oxidative modifications in atherosclerosis. *Physiol Rev*; 84:1381-1478.
- [8] Mukamal KJ, Maclure M, Muller JE, Sherwood JB, Mittleman M.A. 2002. Tea consumption and mortality after acute myocardial infarction. *Circulation*; 105:2476-2481
- [9] Knekt P, Jarvinen R, Reunanen A, Maatela J. 1996. Flavonoid intake and coronary mortality in Finland: a cohort study. *BMJ*; 312:478-481
- [10] Geleijnse JM, Launer LJ, van der Kuip DAM, Hofman A, Witteman JCM. 2002. Inverse association of tea and flavonoid intakes with incident myocardial infarction: the Rotterdam Study. *Am J Clin Nutr*; 75(5):880-886
- [11] Leenen R, Roodenburg AJ, Tjiburg LB, Wiseman SA. 2000. A single dose of tea with or without milk increases plasma antioxidant activity in humans. *Eur J Clin Nutr*; 54:87-92
- [12] Bliss RM. 2003. Brewing up the latest tea research. *Agric Res*; 51:10-13.

- [13] Carlson, JR, Bauer Vincent A, Limburg PJ, Wilson T. 2007. Reading the tea leaves: anticarcinogenic properties of (-)-Epigallocatechin-3-Gallate. *Mayo Clinic Proceedings*; 82(6):725-732
- [14] Frei B, Higdon JV. 2003. Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. *J Nutr*; 133:3275S-3284S.
- [15] Ransod Superoxide dismutase. RANDOX Laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT29 4QY.
- [16] Ohkawa H, Ohishi N, Yagi K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*; 95:351-358.
- [17] Jeong WI, Jeong, DH, Do, SH, Kim, YK, Park, HY, Kwon, OD, Kim, TH, Jeong, KS. 2005 Mild hepatic fibrosis in cholesterol and sodium cholate diet-fed rats. *J Vet MedSci*; 67:235-242
- [18] Horton JD, Cuthbert JA, Spady DK. 1995. Regulation of hepatic 7 alpha-hydroxylase expression and response to dietary cholesterol in the rat and hamster. *J Biol Chem*; 270:5381-5387
- [19] Pellizzon MA. 2008. Diet- induced atherosclerosis/hypercholesterolemia in rodent models. *Res Diets*. 1-3
- [20] Daley SJ, Klemp KF, Guyton JR, Rogers KA. 1994. Cholesterol-fed and casein-fed rabbit models of atherosclerosis. Part 2: Differing morphological severity of atherogenesis despite matched plasma cholesterol Cholesterol-fed and casein-fed rabbit models of atherosclerosis levels. *Arterioscler Thromb Vasc Biol*; 14:105-141
- [21] Tavidou A, Efthimiadis A, Efthimiadis I, Manolopoulos VG. 2010. Simvastatin-induced changes in circulating oxidized low-density lipoprotein in different types of dyslipidemia. *Heart Vessels*; 25:288-293. DOI 10.1007/s00380-009-1202-x
- [22] Davies MJ, Judd JT, Baer DJ, Clevidence BA, Paul DR, Edwards AJ, Wiseman SA, Muesing RA, Chen SC. 2003. Black tea consumption reduces total and ldl cholesterol in mildly hypercholesterolemic adults. *J Nutr*; 133:3298S-3302S
- [23] Sesso HD, Gaziano JM., Buring JE, Hennekens CH. 1999. Coffee and Tea Intake and the Risk of Myocardial Infarction. *Am J Epidemiol*; 149:162-167
- [24] Serafini M, Ghiselli A, Ferro-Luzzi A. 1996. In vivo antioxidant effect of green and black tea in man. *Eur. J Clin Nutr*; 50:79-85
- [25] Vinson JA, Teufel K, Wu N. 2004. Green and black teas inhibit atherosclerosis by lipid, antioxidant, and fibrinolytic mechanisms. *J Agric Food Chem*. 52(11):3661-5.
- [26] Yang M, Wang C, Chen H. 2001. Green, oolong and black tea extracts modulate lipid metabolism in hyperlipidemia rats fed high-sucrose diet. *J Nutr Biochem*; 12(1):14-20.
- [27] Shrestha S, Ehlers SJ, Lee JY, Fernandez ML, Koo SI. 2009. Dietary green tea extract lowers plasma and hepatic triglycerides and decreases the expression of sterol regulatory element-binding protein-1c mrna and its responsive genes in fructose-fed, ovariectomized rats. *J Nutr*; 139(4):640-645.
- [28] Muramatsu K, Fukuyo M, Hara Y. 1986. Effect of green tea catechins on plasma cholesterol level in cholesterol-fed rats. *J Nutr Sci Vitaminol*; 32(6):613-622.
- [29] Matsumoto N, Okushio K, Hara Y. 1998. Effect of black tea polyphenols on plasma lipids in cholesterol-fed rats. *J Nutr Sci Vitaminol*; 44: 337-342.
- [30] Yang CS, Landau JM. 2000. Effects of tea consumption on nutrition and health. *J Nutr*; 130:2409-2412.
- [31] Kono S, Shinchi K, Wakabayashi K, Honjo S, Todoroki I, Sakurai Y, Imanishi K, Nishikawa H, Ogawa S, Katsurada M. 1996. Relation of green tea consumption to serum lipids and lipoproteins in Japanese men. *J Epidemiol*; 6(3):128-33.
- [32] Raederstoff DG, Schlachter MF, Elste V, Weber P. 2003. Effect of EGCG on lipid absorption and plasma lipid levels in rats. *J Nutr Biochem*; 14:326-332.
- [33] Cabrera C, Artacho R, Gimenez R. 2006. Beneficial effects of green tea—a review. *J Am Coll Nutr*; 25(2):79-99
- [34] Chan PT, Fong WP, Cheung, YL, Huang Y, Ho WKK, Chen Z-Y. 1999. Jasmine green tea epicatechins are hypolipidemic in hamsters (*Mesocricetus auratus*) fed a high fat diet. *J Nutr*; 129:1094-1101.
- [35] Nagao T, Komine Y, Soga S, Meguro S, Hase T, Tanaka Y, Tokimitsu I. 2005. Ingestion of a tea rich in catechins leads to a reduction in body fat and malondialdehyde-modified LDL in men. *Am J Clin Nutr*; 81:122-129.
- [36] Maron D J, Lu GP, Cai NS, Wu ZG, Li Y H, Chen H, Zhu JQ, Jin XJ, Wouters BC, Zhao J. 2003. Cholesterol-lowering effect of a theaflavin-

- enriched green tea extract: a randomized controlled trial. *Arch Int Med*;163: 1448-1453
- [37] Judd JT, Davies MJ, Baer DJ, Chan SC, Wiseman S, Agarwal S. 2003. Black tea consumption reduces total and LDLcholesterol in mildly hypercholesterolemic subjects. *J. Nutr*; 133: 3298S-3302S
- [38] Reaven PD, Witztum JL 1996. Oxidized low density lipoproteins in atherogenesis: role of dietary modification. *Ann Rev Nutr*; 16: 51–71
- [39] Libby P, Ridker PM, Hansson GK. 2011. Progress and challenges in translating the biology of atherosclerosis. *Nat*; 473:317-325 doi:10.1038/nature10146
- [40] Loke WM, Proudfoot JM, Hodgson JM, McKinley AJ, Hime N, Magat, Stocker R, Croft KD. 2010. Specific dietary polyphenols attenuate atherosclerosis in apolipoprotein e-knockout mice by alleviating inflammation and endothelial dysfunction. *Arterioscler Thromb Vasc Biol*; 30:749-757
- [41] Fki I, Bouaziz M, Sahnoun Z, Sayadi S. 2005. Hypocholesterolemic effects of phenolic-rich extracts of Chemlali olive cultivar in rats fed a cholesterol-rich diet. *BioorgMed Chem*; 3: 5362–5370
- [42] Kaur GK, Meena C. 2013. Evaluation of anti-hyperlipidemic potential of combinational extract of curcumin, piperine and quercetin in triton-induced hyperlipidemia in rats. *Sci Int*; 1(3):57-63.
- [43] Rivera L, Moron R, sanchez M, Zarzuelo A, Galisteo M. 2008, Quercetin ameliorates metabolic syndrome and improves the inflammatory status in obese Zucker rats. *Obesity* 16:2081–2087
- [44] Shin HS, Yoo JH, Min TS, Lee K-Y, Choi CY. 2010. The effects of quercetin on physiological characteristics and oxidative stress resistance in olive flounder, *paralichthys olivaceus*. *Asian-Aust J Anim Sci*; 23(5):588-559
- [45] Widowati W, Herlina T, Ratnawati H, Mozef T, Risdian T. 2011. Antioxidant and platelet aggregation inhibitor activities of black tea (*Camellia sinensis* L.) extract and fractions. *Med Plants*; 3(1): 21-26.
- [46] Sung H, Min WK., Lee W, Chun S, Park H, Lee Y-W, Jang S, Lee D-H. 2005. The effects of green tea ingestion over four weeks on atherosclerotic markers. *Ann Clin Biochem*; 42:292–297
- [47] Yang TT, Koo MW. 1997. Hypocholesterolemic effects of Chinese tea. *Pharmacol Res Jun*;35(6):505-12.
- [48] Basu A, Lucas EA. 2007. Mechanisms and effects of green tea on cardiovascular health. *Nutr Rev*; 65(8):361-375
- [49] Bhaskar S, Kumar KS, Krishnan K, Antony H. 2013. Quercetin alleviates hypercholesterolemic diet induced inflammation during progression and regression of atherosclerosis in rabbits. *Nutr*; 29(1):219-229. doi: 10.1016/j.nut.2012.01.019
- [50] Terao J, Kawai Y, Murota K. 2008. Vegetable flavonoids and cardiovascular disease. *Asia Pac J Clin Nutr*; 17(S1):291-293
- [51] Moon JH, Tsushida T, Nakahara K, Terao J. 2001. Identification of quercetin 3-O- β -glucuronide as an antioxidative metabolite in rat plasma after oral administration of quercetin. *Free Radical Biol Med*; 30:1274-1285
- [52] Ohara Y, Peterdon TE, Harrison DG. 1993. Hyper-cholesterolemia increases endothelial superoxide anion production. *J Clin Invest*; 91:2546-2551.
- [53] Williamson G, Barron D, Shimoi K, Terao J. 2005. In vitro biological properties of flavonoid conjugates found in vivo. *Free Radical Res*; 39:457-69
- [54] USDA. 2003. USDA Database for the Flavonoid Contents of Selected Foods. Beltsville: US Department of Agriculture.
- [55] Bors W, Saran M. 1987. Radical scavenging by flavonoid antioxidants. *Free Radic Res Commun*; 2:289-294
- [56] Chen Y-H, Li S-J, Chen Y-L, Liu P-L, Chen J-W. 2006. Anti-inflammatory effects of different drugs/agents with antioxidant property on endothelial expression of adhesion molecules. *Cardiovasc Haematological Disorders-Drug Targets*; 6:279-304
- [57] Rosenson RS .2004. Statins in atherosclerosis: lipid-lowering agents with antioxidant capabilities. *Atherosclerosis*; 173:1–12
- [58] Pereira EC, Bertolami MC, Faludi AA, Sevanian A, Abdalla DS. 2004. Antioxidant effect of simvastatin is not enhanced by its association with alpha-tocopherol in hypercholesterolemic patients. *Free Radic Biol Med*; 37:1440-1448
- [59] Prasad K, Kalra J. 1993. Oxygen free radicals and hypercholesterolemic atherosclerosis: effect of vitamin E. *Am Heart J*; 125(4):135-144.
- [60] Prasad K. 2010. Natural products in regression and slowing of progression of atherosclerosis. *Curr Pharmaceut Biotechnol*; 11:794-800.
- [61] Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC. Vitamin E consumption and the risk of coronary heart disease in men. *N Engl J Med*; 328:1450-1456.
- [62] Velayutham P, Babu A, Liu D. 2008. Green Tea Catechins and Cardiovascular Health: An Update. *Curr Med Chem*; 15(18):1840–1850
- [63] Kleemann R, Verschuren L, Morrison M, Zadelaar S, van Erk MJ, Wielinga PY, Kooistra T. 2011. Anti-inflammatory, anti-proliferative and anti-atherosclerotic effects of quercetin in human in vitro and in vivo models. *Ather*; 218(1):44-52.