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

Wahyu Widowati, Hana Ratnawati, Tjandrawati Mozefis, Dwiyati 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 histopathologyof 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 andquercetin 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 multifactoral 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 myricitin) 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.

Dwiyati 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. Extact 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 40^0C. Our process resulted ethanol extract of black tea 119g (11.9%), The ethanol extract of black tea were stored at 4^oC.

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 (25-27^0C, 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 atheroscherosis, 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 histopatholgy 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, LDLl 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	Lipid profile			
	cholesterol	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. Simvasatin, vitamin E, probucol were similar activity to lower cholesterol level. BTE 300mg/kg BW, quercetin 10mg/kg BW were highest activity to lower TG. Simvasatin, 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 g	117.16±5.28 d	48.62±3.98 a	64.84±4.84 d	411.86±17.57 a	7.96±0.48 f
Negative Control	97.07±4.85 a	75.37±4.63 a	106.49±2.67 e	23.95±3.14 a	703.39±41.95 d	1.18±0.19 a
BTE 450 mg/kg	169.01±8.39 c	102.39±3.75 bc	65.59±2.03 d	53.25±3.99 bc	618.65±16.94 c	6.01±0.62 c
BTE 300 mg/kg	192.73±7.93 ef	99.40±4.43 b	58.14±4.30 bc	54.14±3.93 bc	506.78±44.20 b	7.28±0.26 def
BTE 150 mg/kg	206.01±6.04 fg	112.09±4.03 cd	55.56±2.16 bc	59.75±3.70 cd	401.70±15.39 a	7.59±0.14 ef
Quercetin 15 mg/kg BW	154.15±3.16 b	99.70±4.37 b	66.24±3.02 d	51.59±5.48 b	611.86±16.30 c	4.91±0.30 b
Quercetin 10 mg/kg BW	178.18±5.93 cd	106.12±4.07 bc	60.06±3.42 bcd	58.60±3.49 bcd	506.78±26.39 b	6.76±0.47 cd
Quercetin 5 mg/kg BW	206.01±5.90 fg	110.30±5.26 cd	54.41±2.56 ab	57.58±3.38 bcd	410.17±36.25 a	7.64±0.12 ef
Vitamin E 60 mg/kg BW	186.25±5.90 de	104.48±5.33 bc	59.68±2.10 bcd	55.16±3.86 bc	642.37±20.23 cd	7.28±0.43 def
Simvastatin 2.7 mg/kg BW	190.20±8.95 de	103.28±4.31 bc	61.99±3.77 cd	57.71±1.66 bcd	630.51±25.14 c	7.14±.035 de
Probucol 30 mg/kg BW	184.19±9.03 de	104.78±4.74 bc	60.32±3.71 bcd	52.87±2.63 bc	628.81±31.37 c	7.02±0.4 0 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. Simvasatin, vitamin E, probucol were similar activity to lower cholesterol level. BTE 300mg/kg BW was highest activity to lower triglyceride. Simvasatin, 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 velel. 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.

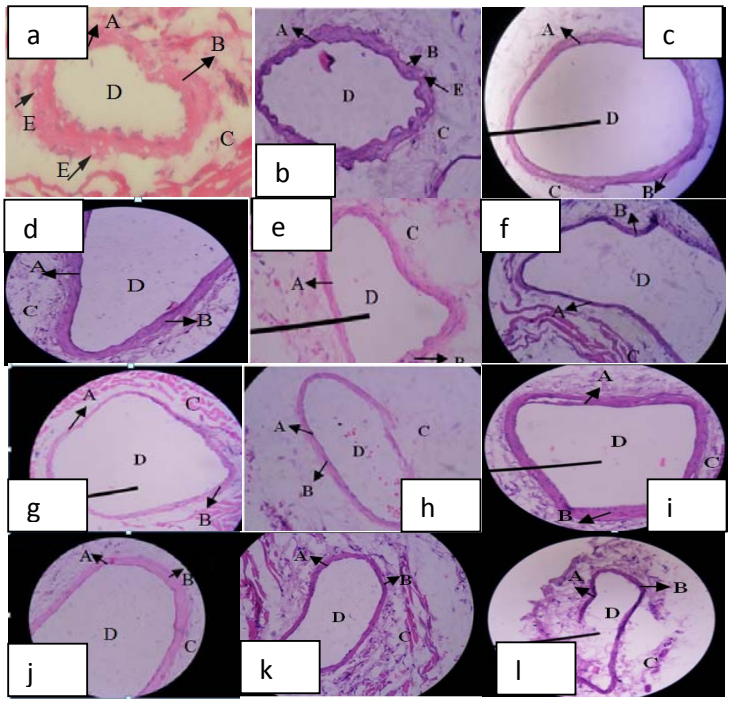

Fig. 1 Histopatholgy of coronary artery of treatment group (400x), (a) : positive control 0-d; (b) positive control 42-d; (c) negative control 42-d; (d) BTE 450mg; (e) BTE 300mg; (f) BTE 150mg; (g) Quercetin 15mg; (h) Quercetin 10mg; (i) Quercetin 5mg; (j) Vitamin E; (k) Simvastatin; (l) Probucol. A = 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-dtreatment 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 ATHEROSCHLEROTIC 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 450 mg/kg	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
BTE 300 mg/kg	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
BTE 150 mg/kg	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
Quercetin 15 mg/kg BW	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
Quercetin 10 mg/kg BW	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
Quercetin 5 mg/kg BW	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
Vitamin E 60 mg/kg BW	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
Simvastatin 2.7 mg/kg BW	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
Probucol 30 mg/kg BW	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

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 antihyperlipedemic 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 cupper 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 metabloits possess xanthin 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 peroxinitrite. 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 lession (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-kB-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.

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