Protective Role of 10-Dehydrogingerdione as Modulator for CETP Activity and HDL Metabolism in Cholesterol Fed Rabbits

10-Dehydrogingerdione Protects Against Atherosclerosis

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Abstract- **Background: Hyperlipidemia represents an important risk factor leading to atherosclerosis additionally life-threatening cardiovascular events such as myocardial infarction and ischemic stroke. Recent evidences referred to phytochemicals and nutraceuticals possessing cholesteryl ester transfer protein (CETP) inhibitory activity as protective agents against lipid-mediated atherosclerosis. Therefore, we studied the effect of 10-dehydrogingerdione, a novel CETP inhibitor isolated from ginger rhizomes on atherosclerosis and its underlying mechanisms based on plasma lipids, lipoprotein metabolism and oxidative stress comparable to the protective effects of atorvastatin (as standard drug) in hypercholesterolemic rabbits. Methods: Twenty four New Zealand male rabbits were randomly divided into four groups (n=6 per group). Three groups were fed an atherogenic diet for 6 weeks. Two groups received either atorvastatin (20 mg / kg body weight orally) or 10-dehydrogingerdione (10 mg / kg body weight orally after isolation and purification) daily for six weeks of treatment. One group received no treatment and served as hypercholesterolemic control group (HCG, positive control) and another one was fed normal diet and used as negative control. Blood and tissues (liver, aorta) samples were collected after six weeks for biochemical and histological analysis. Results: Rabbits fed high cholesterol diet produced a significant dyslipidemia, oxidative stress, and finally atherogenesis progression. These finding evidenced by remarkable increase in serum lipids (TC), non HDL lipoprotein (LDL-C, apo B expression) and decrement of HDL lipoprotein (HDL-C, apo-A1, apo-AII). In addition, increase in CETP level and expression in plasma and liver, MDA, ox-LDL, dense and number of foam cells of aorta was marked. Treatment of hypercholesterolemic rabbits with either of 10 -dehydrogingerdione or atorvastatin for 6 weeks successfully decrease non HDL lipoproteins, aortic foam cell number, increase protective HDL lipoprotein, its apolipoproteins and produced inhibitory effect on CETP. However, 10-dehydrogingerdione exerted better effect than atorvastatin on serum HDL-C ($p<0.001$), CETP mass ($p<0.001$), CETP expression ($p<0.05$), apo A-1 and apo A-II ($p<0.01$), more reduction in the density, number of foam cells and improvement of the intimal lesions of aorta. Conclusions: 10-dehydrogingerdione may provide a protective effect against atherosclerosis compared to atorvastatin. This effect may be through reduction of foam cell number, its potential CETP inhibition, increase HDL-C and wards off oxidation in hypercholesterolemic rabbits.**

Keywords- Hypercholesterolemia; CETP Inhibition; Apolipoprotein Metabolism; Oxidative stress; Atorvastatin; 10-Dehydrogingerdione

I. INTRODUCTION

Atherosclerosis mostly caused by the accumulation of lipids in the arterial wall is a major factor leading to coronary heart disease [1]. Cholesteryl ester transfer protein (CETP) is a hydrophobic plasma glycoprotein which mediates the transfer and exchange of cholesteryl ester (CE) and triacylglycerol (TAG) between plasma lipoproteins [2]. It plays an important role in HDL-CE and apolipoprotein A-I catabolism [3]. It also promotes the reverse cholesterol transport (RCT) where, peripheral cell cholesterol can be returned to the liver for catabolism [3]. Therefore, it is widely accepted that specific inhibitors of the plasma CETP might be good candidates for developing effective therapeutic agents for the treatment of atherosclerotic cardiovascular diseases [4]. Currently, statins that were first discovered as a natural metabolite in *Aspergillus terreus* are the most widely prescribed drug to lower plasma cholesterol levels [5]. However, there is widespread interest in establishing alternative non-pharmacological ways to manage cholesterol based on natural dietary compounds, for reducing atherosclerosis risk [6, 7].

Ginger (*Zingiber officinale* Roscoe), a member of the Gingiberaceae family, is a medicinal plant that has been used worldwide for several kinds of ailments. Numerous compounds have been isolated and identified from ginger such as gingerols, shogaols and paradols [8]. Ginger has received extensive attention due to its pharmacological actions, including anti-inflammatory [9], antitumor [10], anti-apoptotic [11] and antimicrobial [12] actions of its constituents. Recently, Kim et al. [13] discover new inhibitors of CETP isolated from the extract of rhizomes of *Zingiber officinale* and describe the isolation, structure, and CETP inhibitory activity.

Therefore, this study investigates the effect of, 10-dehydrogingerdione, a natural component isolated from ginger on

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atherosclerosis and its underlying mechanisms based on plasma lipids, lipoprotein metabolism and oxidative stress comparable to the protective effects of atorvastatin in hypercholesterolemic rabbits.

II. MATERIALS AND METHODS

A. *Extraction, Isolation and Characterization of 10-Dehydrogingerdione*

The fresh rhizomes of *Zingiber officinalis* Roscoe was purchased from herbal market, Cairo, Egypt, and identified by Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University. The isolation, purification and characterization of 10-dehydrogingerdione was carried out as previously described [13, 14]. In brief, the rhizomes were cut into small pieces then soaked in methanol at room temperature for 3 days. The obtained extract was then filtered and concentrated under reduced pressure at 45°C to obtain a dark brown residue. The latter was suspended in water and was separated into two fractions using n-hexane as the non-aqueous phase. After the organic layer was collected and concentrated, the residue, was subjected to silica gel chromatography (Merck, silica gel 60, 230-400 mesh, 250 g) with a gradient of n-hexane-ethyl acetate (10:0, 9:1, 8:2, 7:3, 6:4 and 5:5 v/v, 1000 ml each) to obtain a yellowish solid. For further purification, fraction 5 was subjected to gas-liquid chromatography-mass spectrometry (GLC-MS) analysis using Gas Chromatograph/Mass Spectrometer (GC/MS) model Clarus 600 (PerkinElmer Life and Analytical Sciences, USA) under certain operating conditions to identify the isolated 10-dehydrogingerdione. Fraction 5 from chromatographic purification of ginger extract corresponds to 10-dehydrogingerdione [14].

B. Animals

Twenty four New Zealand male rabbits, of average weight 1.75 ± 0.25 kg were purchased from the animal farm, Faculty of Agriculture, Zagazig University. Rabbits were housed in stainless steel cages at a temperature (21–23°C) and light controlled room with a 12-h light/dark cycle and ambient humidity (50–60%). Rabbits were fed commercially available rabbit chow diet and water ad libitum. Experimental design and animal handling were performed according to the guidelines of the Ethical Committee of the Faculty of Pharmacy, Zagazig University for animal use.

C. Experimental Design

After one week of acclimatization, rabbits were randomly divided into four groups (n=6 per group). Three groups were fed an atherogenic diet for 6 weeks which consists of normal diet supplemented with 0.2% cholesterol dissolved in coconut oil [15]. Both cholesterol and coconut oil were supplied by EL-Gomhouria CO, Egypt. The first group received no treatment and served as hypercholesterolemic control group (HCG). The other two groups received either atorvastatin (ATOR) (20 mg / kg body weight orally, Lipinorm ®, Sigma Co., Egypt) [16, 17] or 10-dehydrogingerdione (10-DHGD) (10 mg / kg body weight orally) daily for six weeks of treatment. Prior to administration, both atorvastatin and 10-dehydrogingerdione were freshly suspended in distilled water using gum acacia and were orally administered. Rabbits from normal group (NG) were fed normal diet contains 18% pure protein, 2.88% pure fats, and 10.5% pure fibers, supplied from the Faculty of Agriculture, Zagazig University, Zagazig, Egypt.

D. Blood Sampling

At the end of experimental period (6 weeks), blood samples were collected from a marginal ear vein of fasted rabbits (12 h). Sera were prepared by centrifugation of blood samples at 5000xg for 10 min and were divided into aliquots. The first one was processed instantly for the determination of lipogram pattern while the second was stored at -20°C for determination of CETP mass later.

E. Tissue Collection

Following blood collection, animals were scarified; liver and aorta were removed instantly, rinsed with cold normal saline and dried with filter paper. Liver specimen was quickly frozen in liquid nitrogen (-170°C) and stored at -20°C for determination of malondialdehyde (MDA) and gene expression of apo A-I, apo A-II, apo B, and CETP.

Aortic specimen was kept in 10% formalin–saline at 4°C for at least one week (1ry fixation); then the specimens were dehydrated with a series of ascending grade ethanol from 75 to 100%. Tissues were placed thereafter in xylol and embedded in paraffin. Cross-sections of about 2 mm thickness were sliced using a microtome (Leica RM 2155, England) then processed on slides and stained with hematoxylin and eosin (H&E) stain [18] for microscopical examination.

F. Analytical Methods

Total cholesterol, Triglycerides and HDL-C were determined colorimetrically using assay kits supplied by Spinreact Co., Spain. LDL-C was calculated according to Friedewald formula: LDL-C (mg/dl) = TC – [HDL-C + TAG/5] [19] and atherogenic index was calculated from the ratios (LDL-C/HDL-C and TC/HDL-C). CETP mass was determined by sandwitch ELISA using rabbit ELISA kits supplied by Uscn Life Science Inc, China.

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Lipid peroxidation was estimated by measuring the formation of thiobarbituric acid reactive substances, expressed in terms of MDA, according to the method of [20] using Bio-diagnostic Kits, Egypt, following the instructions of the manufacturers.

G. RNA Isolation and RT-PCR Assay for Apo A-I, Apo A-II, CETP and Apo B Genes

For the detection of apo A-I, apo A-II, CETP and apo B genes by real-time polymerase chain reaction (RT-PCR), RNA was extracted using SV Total RNA isolation system (Promega, Madison, WI, USA), reverse transcribed into cDNA and amplified by PCR using RT-PCR kit (Stratagene, USA). The oligonucleotide sequences of forward and reverse primers are as shown in Table 1. The amplification reactions were performed in a 50µl final volume, with thermal cycling conditions of 2 min at 50°C, 10 min at 95°C, and 40 cycles of 15 s at 95°C, 30 s at 60°C 30 s at 72°C, and 10 min at 72°C. Cycle threshold (Ct) data were normalized using GAPDH, which was stably expressed across all experimental groups. Relative gene expression was calculated using the $-2^{\Delta\Delta Ct}$ method [21].

TABLE 1 SEQUENCE OF THE PRIMERS USED FOR REAL-TIME PCR

Gene	Primer sequence	Annealing Temp. (°C)	Product size (bp)
Apo A-I	Forward primer: 5'-TGTGTATGTGGATGCGGTCA-3' Reverse primer: 5'-ATCCCAGAAGTCCCGAGTCA-3'	61°C	172bp
Apo A-II	Forward primer: 5'-AATGGTCGCACTGCTGGTAA-3' Reverse primer: 5'-TTGGCCTTCTCCACCAAATC-3'	60°C	141bp
CETP	Forward primer: 5'-GGTTGGGCATCAATCAGTCT-3' Reverse primer: 5′-CAGCCATGATGTTGGAGATG-3′	60°C	445bp
Apo B	Forward primer: 5′-TCCTCAGCAGATTCATGATTATCT-3′ Reverse primer: 5′-AGCATTTTTAGCTTTTCAATGATT-3′	61°C	297bp
GAPDH	Forward primer: 5′-GTCGGTGTGAACGGATTTG-3′ Reverse primer: 5′-AAGATGGTGATGGGCTTCC-3′	61°C	215bp

H. Statistical Analyses

Statistical analyses of data were done by Prism 5, Graph pad, CA, USA. Results were expressed as mean ± standard deviation. Statistical differences were sought using Student's t-test or one way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) post hoc test (if more than two sets of data were being compared). The significance of relationships between variables was calculated by linear regression analysis. Differences were considered significant at a $P<0.05$.

III. RESULTS

A. Effect of 10-Dehydrogingerdione versus Atorvastatin on Plasma Lipids

Rabbits fed high cholesterol diet produced a significant increase ($p<0.001$) in serum TC level along with non significant increase of TAG as compared to normal group (NG). Treatment of hypercholesterolemic rabbits with both of 10-dehydrogingerdione (10-DHGD) and atorvastatin (ATOR) for 6 weeks induced remarkable decrease in serum lipid levels ($p<0.001$). 10-DHGD induced more pronounced effect regarding the above mentioned items as compared to ATOR group (Table 2).

B. Effect of 10-Dehydrogingerdione versus Atorvastatin on Plasma Lipoproteins, CETP Mass and Liver MDA

HDL-C level was significantly reduced in rabbits fed high cholesterol diet, whereas, LDL-C showed marked increase compared to normal rabbits ($p<0.001$). Both 10-DHGD and ATOR significantly increase HDL-C and lower LDL-C compared with HCG ($p<0.001$). The ratios of LDL-C/HDL-C and TC/LDL-C were subsequently increased in HCG compared with NG ($P<0.001$) and they were significantly reduced by 10-DHGD after 6 weeks. Similar effect was induced by ATOR.

High cholesterol diet induced a significant increases (4 fold) in plasma CETP mass as compared to NG ($p<0.001$). Treatment with 10-DHGD and ATOR successfully decrease CETP compared with HCG ($p<0.001$), however, 10-DHGD has more prominent effect compared to ATOR ($p<0.001$). Oxidative stress expressed as MDA content in the liver was exaggerated in HCG for 6 weeks compared to NG ($p<0.001$). Both 10-DHGD and ATOR attenuated and decreased liver MDA content ($P<0.001$) as compared to HCG. These results are summarized in (Table 2).

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TABLE 2 EFFECT OF 10-DEHYDROGINGERDIONE VERSUS ATROVASTATIN ADMINISTRATION ON BLOOD LIPIDS, CETP MASS AND OXIDATIVE STRESS MARKER IN HYPERCHOLESTEROLEMIC RABBITS

Parameters	NG (n=6)	HCG (n=6)	ATOR (n=6)	10-DHGD (n=6)
TC (mg/dl)	132.2 ± 10.70	441.5 ± 55.77$^{\#}$	167.3 ± 23.23*****	132.2 ± 10.70*****
TAG (mg/dl)	69.83 ± 6.080	72.33 ± 8.618	41.0 ± 5.329$^{\#}$*****	33.00 ± 5.177$^{\#}$*****
HDL-C (mg/dl)	32.23 ± 3.618	18.1 ± 0.766$^{\#}$	34.98 ± 1.401*****	41.65 ± 1.555$^{\#}$***** ^{c}
LDL-C (mg/dl)	86.0 ± 12.95	408.9 ± 53.86$^{\#}$	124.2 ± 21.10 *****	83.92 ± 8.560*****
CETP mass (pg/ml)	76.47 ± 3.455	316.0 ± 9.490$^{\#}$	154.3 ± 1.814*****	127.1 ± 2.644*****^{c}
MDA (nmol/g.tissue)	77.5 ± 2.51	115.7 ± 5.164$^{\#}$	83.67 ± 1.751*****	80.67 ± 3.445*****
TC/HDL-C	3.1 ± 0.268	24.3 ± 2.18 $^{\#}$	4.769 ± 0.49*	3.169 ± 0.656 *****
LDL-C/HDL-C	1.66 ± 0.276	22.5 ± 2.15 $^{\#}$	3.54 ± 0.475*****	2.011 ± 0.136 *****

NG: rabbits were fed a normal diet; HCG: rabbits were fed a high cholesterol diet for 6 weeks and did not receive drug treatment; ATOR group: rabbits were fed a high cholesterol diet and received atorvastatin (20 mg/kg/d) and 10-DHGD group: rabbits were fed a high cholesterol diet and received 10-dehydrogingerdione (10 mg/kg/d). Data are presented as the mean ± SD, n = 6. TC: Total cholesterol; TAG: Triacylglycerol; HDL-C: High-density lipoprotein; LDL-C: Low-density lipoprotein; CETP: Cholesteryl ester transfer protein; MDA: Malondialdehyde. $^{\#}$p<0.001 vs NG, *****p<0.001 vs HCG and

^{c} p<0.001 vs ATOR

C. Effect of 10-Dehydrogingerdione versus Atorvastatin on Liver Gene Expression of Apo A-I, Apo A-II, Apo B and CETP

Gene expression of apo A-I and apo A-II was significantly downregulated in hypercholesterolemic rabbits meanwhile, apo-B and CETP gene expression was upregulated as compared to NG (p<0.001). Treatment with either ATOR or 10-DHGD for 6 weeks induced a significant increase in the hepatic gene expression of both apo A-I and apo A-II compared with HCG (p<0.001).

On the other hand, ATOR and 10-DHGD treatment significantly decreased apo B and CETP gene expression (p<0.001) as compared to HCG. However, 10-DHGD exerted better effect than ATOR on apo A-I, apo A-II (p<0.01) and CETP gene expression (p<0.05). All these results are summarized in Fig. 1.

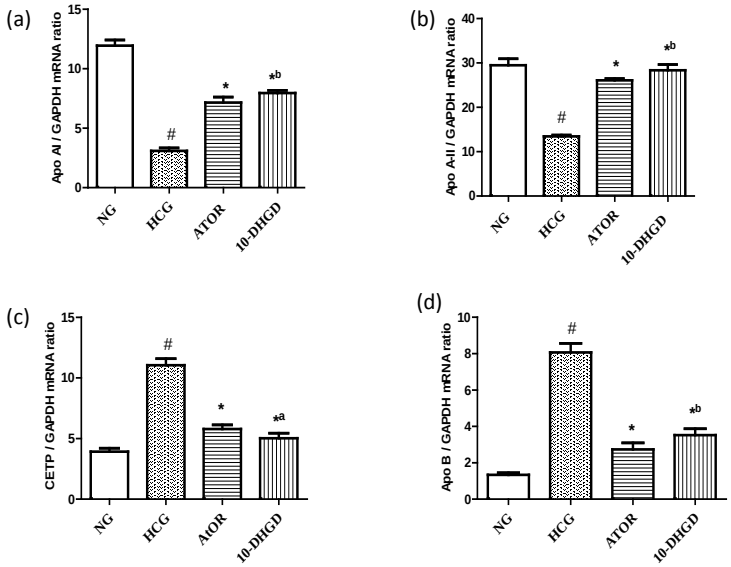

Fig. 1 Hepatic gene expression of apo A-I, apo A-II, CETP and apo B relative to GAPDH in hypercholesterolemic rabbits and daily oral concurrent administration of atorvastatin and 10- dehydrogingerdione. NG: rabbits were fed a normal diet; HCG: rabbits were fed a high cholesterol diet for 6 weeks and did not receive drug treatment; ATOR group: rabbits were fed a high cholesterol diet and received atorvastatin (20 mg/kg/d) and 10-DHGD group: rabbits were fed a high-cholesterol diet and received 10-dehydrogingerdione (10 mg/kg/d). Data are presented as the mean ± SD, n = 6. Apo AI: Apolipoprotein AI; Apo AII: Apolipoprotein AII; Apo B: Apolipoprotein B; CETP: Cholesteryl ester transfer protein; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

$^{\#}$ p<0.001 vs NG, *p<0.001 vs HCG and ^{a} p<0.05, ^{b} p<0.01 vs ATOR.

D. Histopathological Results

The microscopical examination of the aorta of normal rabbits (Fig. 2A), showed simple squamous epithelium lining the interior surface of the vessel with intact prominent internal elastic lamina, normal subendothelial layer of loose connective tissue and tunica media is formed of smooth muscle fibers and elastic fibers. The aorta from hypercholesterolemic rabbits (Fig. 2B), shows separation of the endothelial cell denoting an intimal injury. Neointimal lesions in cholesterol-fed rabbits were

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heterogeneous and composed of foam cells, smooth muscle cells and collagen fibers. The aorta from ATOR-treated rabbits (Fig. 2C), showed a reduction in the density as well as the number (Fig. 3) of foam cells reflecting an improvement of the intimal lesions. The aorta from 10-DHGD-treated rabbits (Fig. 2D), exhibited a more reduction in the density, number (Fig. 3) of foam cells and showed also an improvement of the intimal lesions.

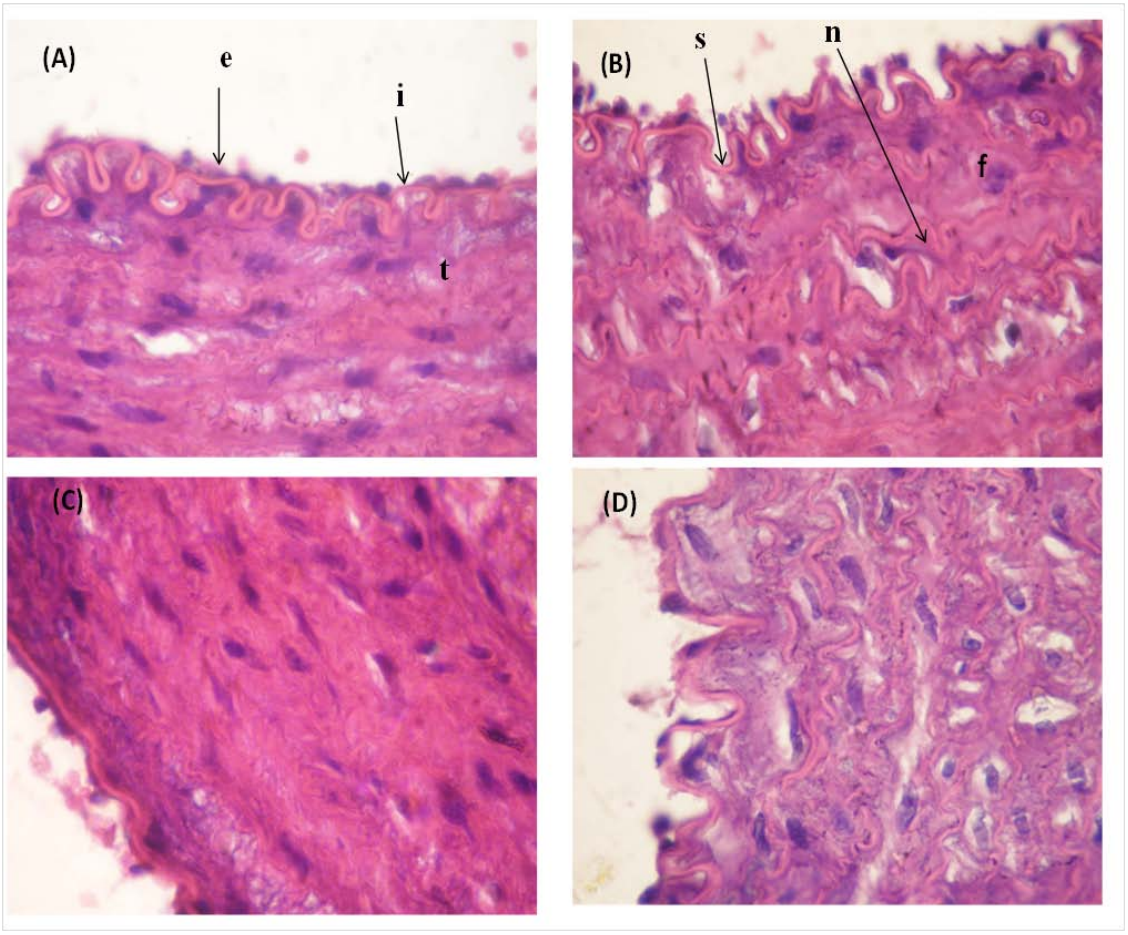

Fig. 2 (A) Sections in aorta of normal rabbits (NG). (B) Sections in aorta from hypercholesterolemic rabbits (HCG). (C) Sections of the aorta from ATOR-treated rabbits. (D) Sections of the aorta from 10-DHGD treated rabbits. Hematoxylin and eosin staining of the aorta of normal rabbits (A) showing simple squamous epithelium (arrows with letter e) lining the interior surface of the vessel with intact prominent internal elastic lamina (arrows with letter i), normal subendothelial layer of loose connective tissue and tunica media (arrows with letter t) is formed of smooth muscle fibers and elastic fibers. (B) The aorta from hypercholesterolemic rabbits showing separation (arrows with letter s) of the endothelial cell denoting an intimal injury. Neointimal lesions (arrows with letter n) in cholesterol-fed rabbits were heterogeneous and composed of foam cells (arrows with letter f), smooth muscle cells and collagen fibers. (C) The aorta from ATOR-treated rabbits showing a reduction in the density as well as the number of foam cells and improvement of the intimal lesions. (D) The aorta from 10-DHGD-treated rabbits showing a more reduction in the density, number of foam cells and improvement of the intimal lesions. H & E X1000.

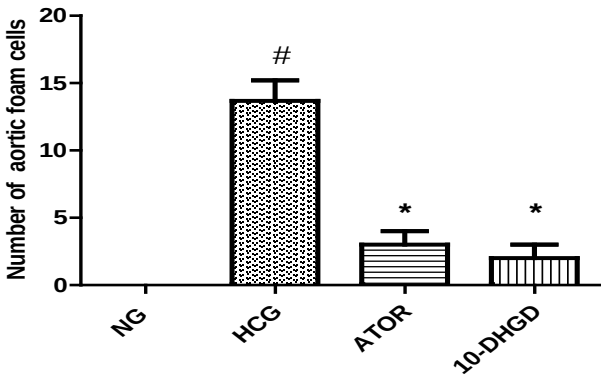

Fig. 3 Number of aortic foam cells in different groups. NG: rabbits were fed a normal diet; HCG: rabbits were fed a high cholesterol diet for 6 weeks and did not receive drug treatment; ATOR group: rabbits were fed a high cholesterol diet and received atorvastatin (20 mg/kg/d) and 10-DHGD group: rabbits were fed a high cholesterol diet and received 10-dehydrogingerdione (10 mg/kg/d). Data are presented as the mean ± SD, n = 6. $^{\#}$ $p<0.001$ vs NG, *$p<0.001$ vs HCG.

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E. Biochemical Correlation

Using the combined data from week 6 for all groups revealed that the plasma CETP mass was correlated negatively with apo A-I and apo A-II [r = -0.9465 and -0.9772 respectively, p<0.0001, n=24] and positively with hepatic gene expression of CETP [r=0.9856, p<0.0001, n=24], MDA [r = 0.9671, p<0.0001, n=24] and atherogenic index values [r = 0.9631 and 0.9604 respectively, p<0.0001, n=24] Fig. 4.

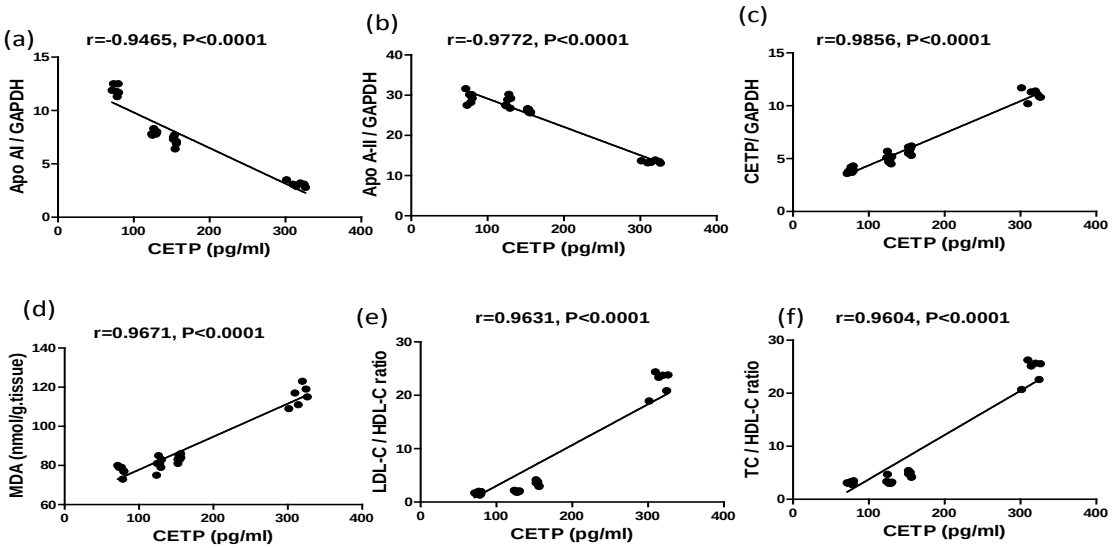

Fig. 4 Correlations between CETP mass and apo A-I, apo A-II, hepatic gene expression of CETP, MDA and atherogenic index values. CETP was strongly correlated negatively with apo A-1, apo A-II and positively with hepatic gene expression of CETP, MDA and atherogenic index values.

IV. DISSCUSION

Inhibition of CETP provides a useful strategy to raise HDL-C, the protective lipoprotein fraction in serum that augments the anti-inflammatory, antithrombotic, and antioxidative potential [22]. Previous studies for human having CETP deficiency and other animal studies have suggested that increased HDL-C levels are attributed to reduced CETP activity. The latter impedes reverse cholesterol transport, thereby perhaps may be proatherogenic [23].

Therefore we first discuss the effect of feeding a high cholesterol diet on lipids and lipoprotein metabolism with a subsequent focus on atherosclerosis. The main goal is to test the effect of 10-dehydrogingerdione, a natural component isolated from ginger on atherosclerosis and its underlying mechanisms based on plasma lipids, lipoprotein metabolism and oxidative stress using atorvastatin as standard drug in hypercholesterolemic rabbits.

In this study, hypercholesterolemia was induced in experimental rabbits where lipoprotein profile of rabbits is nearly similar to humans than to rodents, as CETP mass and activity are present in rabbit plasma and rabbits represent a well-established model for atherosclerosis. In our model, 0.2% cholesterol dissolved in coconut oil was mixed with rabbit chow and fed for 6 weeks to induce hypercholesterolemia that was characterized by an increase in both total cholesterol and LDL-C cholesterol along with HDL-C decrease, no change was observed in TG and in agreement with reported study [24].

Recorded results showed that dietary cholesterol downregulated hepatic apo A-I and apo A-II gene expression by affecting their transcription rates, additionally significant increase in CETP mass and MDA were also reported in HCG.

The biosynthesis of HDL is complex process which involves the synthesis and secretion of the major protein components of HDL followed by the largely extracellular acquisition of lipids (phospholipids and cholesterol) and finally the assembly and generation of the mature HDL particle. The major HDL apolipoproteins are apo A-I, apo A-II, and both are required for normal HDL biosynthesis [25]. Previous study indicated that hepatic over-expression of apo A-I significantly raises HDL-C levels and inhibits the progression of and even regresses atherosclerosis in mice [26]. Moreover, gene deletion of apo A-II in mice can markedly reduces HDL-C levels [27] where apo A-II is also required for normal HDL biosynthesis and metabolism.

Cholesteryl ester transfer protein (CETP) is known to promote reverse cholesterol transport by transferring CE from HDL to TG-rich lipoproteins (chylomicron, VLDL and LDL) in exchange for TG. This would result in decreasing circulating HDL-C levels and increasing cholesterol content of atherogenic lipoproteins. Therefore, CETP will be either pro- or anti-atherogenic depending on the catabolic mechanism of atherogenic lipoproteins [28]. A strong negative correlation between serum CETP and apo A-I or apo A-II here may add further support for the concept of its proatherogenic pathway.

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In this study, the consequences of CETP inhibition by 10-dehydrogingerdione was previously reported ex-vivo [13]. Present study showed that 10-dehydrogingerdione reduced total as well as non HDL-C and raised HDL-C, additionally; significant and remarkable attenuation for lipoprotein metabolism can augment the anti-atherogenic properties. Similar effects on lipid profile were also reported by ginger extract and other synthetic CETP inhibitors [29, 30]. Previous report in human revealed a strong correlation between CETP mass and activity in diabetic patients [31] and in patients with hypercholesterolemia and combined hyperlipidemia [32].

Atorvastatin induced similar, but less noteworthy anti-atherogenic effects than that of 10-dehydrogingerdione. The ability of atorvastatin to reduce CETP mass and to moderately increase HDL-C was reported before [33]. The reduction of CETP mass in all groups studied is well-correlated with the hepatic gene expression of CETP and with the anti-atherogenic lipid profile suggesting a possible anti-atherogenic effect of 10-dehydrogingerdione through CETP inhibition. In accordance, CETP was strongly correlated negatively with apo A-I, apo A-II and positively with hepatic gene expression of CETP and atherogenic index.

Hypocholesterolemic effects of 10-dehydrogingerdione, a constituent of ginger extract, may be through its activity on hepatic cholesterol-7α-hydroxylase, which catalyses the conversion of hepatic cholesterol to bile acids, an important pathway for cholesterol elimination from the body [34]. Accordingly, reduction of intracellular cholesterol may lead to downregulate CETP gene expression as a result of the presence of cholesterol responsive element in the promoter of the human CETP gene contributing to decreased circulating levels of CETP [35]. Previous study, [36] similarly showed that plasma CETP was found to be increased in hypercholesterolemic individuals.

As a result of decreased abundance of CETP mRNA in the liver and CETP mass, the plasma CETP activity is also decreased [37]. Since CETP removes CE from HDL in exchange for TG, low CETP in turn results in high HDL, where CE tends to accumulate in HDL leading to a reduction in its catabolism along with significant decrease of LDL-C. Here LDL particles will contain lesser amounts of cholesterol and in turn an increased rate of clearance from the plasma [29]. These results are also in agreement with previous clinical study where ginger administration decreased LDL-C and increased HDL-C levels in hyperlipidemic patients [38].

Besides upregulation of apo A-I and apo A-II expression, it was reported that CETP inhibition increased plasma concentrations of HDL apo A-I by delaying apo A-I catabolism, the latter is likely attributable to CE enrichment of HDL where its particle acquire increased size [39].

Kastelein et al. [40], demonstrated that increased plasma HDL-C levels are widely associated with decreased risk of atherosclerosis and cardiovascular disease. Atherosclerotic lesion development could be inhibited through manipulation of reverse cholesterol transport components such as HDL-C, CETP and apo A-I [41].

In contrast, apo B mRNA levels were lowered by 10-dehydrogingerdione treatment, similar to atorvastatin treatment. Apolipoprotein B (Apo B) is synthesized and released from the liver, where it forms a 1:1 lipoprotein complex with LDL-C; hence, apo B decrease indicates constitutively lower LDL-C levels.

Previous reports have demonstrated that the overproduction of reactive oxygen species (ROS) or increased oxidative stress is a major mechanism involved in the pathogenesis of vascular endothelial dysfunction, the initiation and progression of atherosclerosis and its adverse events [42, 43].

Present study showed that CETP inhibition with 10-dehydrogingerdione retards the progression of atherosclerosis in rabbits, perhaps as a result of the reduction of MDA content of liver and Ox-LDL of plasma in hypercholesterolemic rabbits. These finding may be related to ginger where *Zingiber officinale* contains a number of antioxidants such as as gingirols, shogaols, beta-carotene and ascorbic acid which inhibit lipid peroxidation and attribute to free radical scavenging activity [44, 45]. In consequent, there is a strong correlation between either MDA or atherogenic index and CETP mass.

Ox-LDL is considered to be a key factor of initiating and accelerating atherosclerosis. It enhances cellular cholesterol accumulation and foam cell formation, the hallmark of early atherosclerosis. Both 10-dehydrogingerdione and atorvastatin exhibited antioxidant effect by lowered plasma ox-LDL in treated rabbits compared with rabbits in HCG group [14]. Fuhrman and colleges [46] provided evidence that ginger extract can reduce macrophage-mediated oxidation of LDL, reduce uptake of ox-LDL by macrophages, reduce oxidative state of LDL and its aggregation. The previous finding is consistent with reduction in the density and the number of foam cells reflecting an improvement of the intimal lesions in treated groups. However, 10-dehydrogingerdione exhibited a better and more reduction of foam cell number than atorvastatin group.

V. CONCLUSIONS

One critical sequel of feeding high cholesterol diet in rabbits is increased mRNA expression of CETP, apo B gene, MDA and down-regulated both apo A-I and apo A-II which are implicated in the development of atherosclerosis. 10-dehydrogingerdione may provide a protective effect compared to atorvastatin. This effect may be through reduction in the density as well as the number of foam cells and improvement of the intimal lesions of aorta, its potential CETP inhibition,

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raises HDL-C and wards off oxidation in hypercholesterolemic rabbits. Lastly further clinical studies in the future regarding 10-dehydrogingerdione are highly recommended.

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REFERENCES

[1] G. J. Reimers, C. L. Jackson, J. Rickards, P.Y. Chan, J. S. Cohn, K. -A. Rye, P. J. Barter and K. J. Rodgers, "Inhibition of rupture of established atherosclerotic plaques by treatment with apolipoprotein AI," Cardiovascular Research, vol. 91(1), pp. 37-44, 2011.

[2] S. Simonelli, D. Baldassarre, M. Amato, S. Catelnuovo, B. Frigerio, A. Ravani, D. Sansaro, F. Veglia, G. Franceschini and E. Tremoli, "Plasma cholesteryl ester transfer protein (Cetp) and carotid intima-media thickness in european indivduals at high cardiovascular risk," Giornale Italiano Dell'Aterosclerosi, vol. 3(4), pp. 79-79, 2012.

[3] Z. Huang, A. Inazu, A. Nohara, T. Higashikata and H. Mabuchi, "Cholesteryl ester transfer protein inhibitor (JTT-705) and the development of atherosclerosis in rabbits with severe hypercholesterolaemia," Clinical Science, vol. 103(6), pp. 587-594, 2002.

[4] P. Parini and L. L. Rudel, "Is there a need for cholesteryl ester transfer protein inhibition?" Arteriosclerosis, Thrombosis, and Vascular Biology, vol. 23(3), pp. 374-375, 2003.

[5] Program, T. R. o. t. N. C. E., "Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult treatment panel III) final report," Circulation, vol. 106, pp. 3143-3421, 2002.

[6] B. B. Aggarwal, "Targeting inflammation-induced obesity and metabolic diseases by curcumin and other nutraceuticals," Annual Review of Nutrition, vol. 30, pp. 173, 2010.

[7] J. Epstein, I. R. Sanderson and T. T. MacDonald, "Curcumin as a therapeutic agent: the evidence from in vitro, animal and human studies," British Journal of Nutrition, vol. 103(11), pp. 1545-1557, 2010.

[8] B. H. Ali, G. Blunden, M. O. Tanira and A. Nemmar, "Some phytochemical, pharmacological and toxicological properties of ginger (< i> Zingiber officinale</i> Roscoe): A review of recent research," Food and Chemical Toxicology, vol. 46(2), pp. 409-420, 2008.

[9] R. Grzanna, L. Lindmark and C. G. Frondoza, "Ginger-an herbal medicinal product with broad anti-inflammatory actions," Journal of Medicinal Food, vol. 8(2), pp. 125-132, 2005.

[10] S. Sang, J. Hong, H. Wu, J. Liu, C. S. Yang, M. -H. Pan, V. Badmaev and C. -T. Ho, "Increased growth inhibitory effects on human cancer cells and anti-inflammatory potency of shogaols from Zingiber officinale relative to gingerols," Journal of Agricultural and Food Chemistry, vol. 57(22), pp. 10645-10650, 2009.

[11] C. -Y. Chen, C. -J. Tai, J. -T. Cheng, J. -J. Zheng, Y. -Z. Chen, T. -Z. Liu, S. -J. Yiin and C.-L. Chern, "6-dehydrogingerdione sensitizes human hepatoblastoma Hep G2 cells to TRAIL-induced apoptosis via reactive oxygen species-mediated increase of DR5," Journal of Agricultural and Food Chemistry, vol. 58(9), pp. 5604-5611, 2010.

[12] G. Jagetia, M. Baliga and P. Venkatesh, Ginger (Zingiber officinale Rosc.), "a dietary supplement, protects mice against radiation-induced lethality,Mechanism of action," Cancer Biotherapy & Radiopharmaceuticals, vol. 19(4), pp. 422-435, 2004.

[13] S. -Y. Choi, G. -S. Park, S. Y. Lee, J. Y. Kim and Y. K. Kim, "The conformation and CETP inhibitory activity of [10]-dehydrogingerdione isolated from Zingiber officinale," Archives of Pharmacal Research, vol. 34(5), pp. 727-731, 2011.

[14] M. M. Elseweidy, F. R. Abdallah, N. N. Younis, S. Aldohmy and H. M. Kassem, "10-Dehydrogingerdione raises HDL-cholesterol through a CETP inhibition and wards off oxidation and inflammation in dyslipidemic rabbits," Atherosclerosis, vol. 231(2), pp. 334-340, 2013.

[15] B. Madhumathi, M. Venkataranganna, S. Gopumadhavan, M. Rafiq and S. Mitra, "Induction and evaluation of atherosclerosis in New Zealand white rabbits," Indian Journal of Experimental Biology, vol. 44(3), pp. 203, 2006.

[16] G. H. Heeba and M. I. Abd-Elghany, "Effect of combined administration of ginger (< i> Zingiber officinale</i> Roscoe) and atorvastatin on the liver of rats," Phytomedicine, vol. 17(14), pp. 1076-1081, 2010.

[17] I. I. Siempos, N. A. Maniatis, P. Kopterides, C. Magkou, C. Glynos, C. Roussos and A. Armaganidis, "Pretreatment with atorvastatin attenuates lung injury caused by high-stretch mechanical ventilation in an isolated rabbit lung model," Critical Care Medicine, vol. 38(5), pp. 1321-1328, 2010.

[18] R. A. Drury and E. A. Wallington, Histological techniques 5th ed. Oxford University press, Oxford, N.Y., Toronto, pp. 27-29, 1980.

[19] W. T. Friedewald, R. I. Levy and D. S. Fredrickson, "Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge," Clinical Chemistry, vol. 18(6), pp. 499-502, 1972.

[20] H. Ohkawa, N. Ohishi and K. Yagi, "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction," Analytical Biochemistry, vol. 95(2), pp. 351-358, 1979.

[21] T. D. Schmittgen and K. J. Livak, "Analyzing real-time PCR data by the comparative CT method," Nature Protocols, vol. 3(6), pp. 1101-1108, 2008.

[22] H. Hirata, S. Segawa, M. Ozaki, N. Kobayashi, T. Shigyo and H. Chiba, "Xanthohumol prevents atherosclerosis by reducing arterial cholesterol content via CETP and apolipoprotein E in CETP-transgenic mice," PloS One, vol. 7(11), pp. e49415, 2012.

DOI:10.5963/BER0302001

[23] L. A. Morehouse, E. D. Sugarman, P. -A. Bourassa, T. M. Sand, F. Zimetti, F. Gao, G. H. Rothblat and A. J. Milici, "Inhibition of CETP activity by torcetrapib reduces susceptibility to diet-induced atherosclerosis in New Zealand White rabbits," Journal of Lipid Research, vol. 48(6), pp. 1263-1272, 2007.

[24] D. Cavallini, R. Bedani, L. Q. Bomdespacho, R. C. Vendramini and E. A. Rossi, "Effects of probiotic bacteria, isoflavones and simvastatin on lipid profile and atherosclerosis in cholesterol-fed rabbits, a randomized double-blind study," Lipids Health Dis, vol. 8(1), pp. 1-8, 2009.

[25] D. J. Rader, "Molecular regulation of HDL metabolism and function,implications for novel therapies," Journal of Clinical Investigation, vol. 116(12), pp. 3090-3100, 2006.

[26] R. K. Tangirala, K. Tsukamoto, S. H. Chun, D. Usher, E. Puré and D. J. Rader, "Regression of atherosclerosis induced by liver-directed gene transfer of apolipoprotein AI in mice," Circulation, vol. 100(17), pp. 1816-1822, 1999.

[27] W. Weng and J. L. Breslow, "Dramatically decreased high density lipoprotein cholesterol, increased remnant clearance, and insulin hypersensitivity in apolipoprotein A-II knockout mice suggest a complex role for apolipoprotein A-II in atherosclerosis susceptibility," Proceedings of the National Academy of Sciences, vol. 93(25), pp. 14788-14794, 1996.

[28] P. N. Durrington and P. Durrington, "Cholesteryl ester transfer protein (CETP) inhibitors," British Journal of Cardiology, vol. 19(3), p. 126, 2012.

[29] S. J. Nicholls, H. B. Brewer, J. J. Kastelein, K. A. Krueger, M. -D. Wang, M. Shao, B. Hu, E. McErlean and S. E. Nissen, "Effects of the CETP inhibitor evacetrapib administered as monotherapy or in combination with statins on HDL and LDL cholesterol," The Journal of the American Medical Association, vol. 306(19), pp. 2099-2109, 2011.

[30] A. Bochem, J. Kuivenhoven and E. Stroes, "The promise of cholesteryl ester transfer protein (CETP) inhibition in the treatment of cardiovascular disease," Current Pharmaceutical Design, vol. 19(17), pp. 3143-3149, 2013.

[31] F. V. van Venrooij, R. P. Stolk, J. -D. Banga, T. P. Sijmonsma, A. van Tol, D. W. Erkelens and G. M. Dallinga-Thie, "Common cholesteryl ester transfer protein gene polymorphisms and the effect of atorvastatin therapy in type 2 diabetes," Diabetes Care, vol. 26(4), pp. 1216-1223, 2003.

[32] F. Tatò, G. L. Vega, A. R. Tall and S. M. Grundy, "Relation between cholesterol ester transfer protein activities and lipoprotein cholesterol in patients with hypercholesterolemia and combined hyperlipidemia," Arteriosclerosis, Thrombosis, and Vascular Biology, vol. 15(1), pp. 112-120, 1995.

[33] S. Rashid, K. D. Uffelman, P. H. R. Barrett and G. F. Lewis, "Effect of atorvastatin on high-density lipoprotein apolipoprotein AI production and clearance in the New Zealand white rabbit," Circulation, vol. 106(23), pp. 2955-2960, 2002.

[34] R. Mahmoud, W. Elnour, D. Costantino, G. Minozzi, F. Minozzi, C. Guaraldi, R. Saraceno, M. Ruzzetti, M. De Martino and L. Di Renzo, "Comparative evaluation of the efficacy of ginger and orlistat on obesity management, pancreatic lipase and liver peroxisomal catalase enzyme in male albino rats," European Review for Medical and Pharmacological Sciences, vol. 17(1), pp. 75-83, 2013.

[35] E. F. Villard, M. -C. Federspiel, C. Cherfils, V. Fesel-Fouquier, E. Bruckert, K. Clement, D. Bonnefont-Rousselot, W. Le Goff, R. Bittar and P. Couvert, Endogenous CETP activity as a predictor of cardiovascular risk, Determination of the optimal range. Atherosclerosis, 2013.

[36] G. J. de Grooth, T. J. Smilde, S. van Wissen, A. H. Klerkx, A. H. Zwinderman, J. -C. Fruchart, J. J. Kastelein, A. F. Stalenhoef and J. A. Kuivenhoven, "The relationship between cholesteryl ester transfer protein levels and risk factor profile in patients with familial hypercholesterolemia," Atherosclerosis, vol. 173(2), pp. 261-267, 2004.

[37] M. -J. Kwon, Y. -S. Song, M. -S. Choi and Y. -O. Song, "Red pepper attenuates cholesteryl ester transfer protein activity and atherosclerosis in cholesterol-fed rabbits," Clinica Chimica Acta, vol. 332(1), pp. 37-44, 2003.

[38] R. Alizadeh-Navaei, F. Roozbeh, M. Saravi, M. Pouramir, F. Jalali and A. A. Moghadamnia, "Investigation of the effect of ginger on the lipid levels," Saudi Med J., vol. 29(9), pp. 1280-1284, 2008.

[39] M. E. Brousseau, M. R. Diffenderfer, J. S. Millar, C. Nartsupha, B. F. Asztalos, F. K. Welty, M. L. Wolfe, M. Rudling, I. Björkhem and B. Angelin, "Effects of cholesteryl ester transfer protein inhibition on high-density lipoprotein subspecies, apolipoprotein AI metabolism, and fecal sterol excretion," Arteriosclerosis, Thrombosis, and Vascular Biology, vol. 25(5), pp. 1057-1064, 2005.

[40] B. J. Arsenault, S. M. Boekholdt and J. J. Kastelein, "Lipid parameters for measuring risk of cardiovascular disease," Nature Reviews Cardiology, vol. 8(4), pp. 197-206, 2011.

[41] A. V. Khera and D. J. Rader, "Future therapeutic directions in reverse cholesterol transport," Current Atherosclerosis Reports, vol. 12(1), pp. 73-81, 2010.

[42] Y. Hou, W. Shao, R. Xiao, K. Xu, Z. Ma, B. H. Johnstone and Y. Du, "Pu-erh tea aqueous extracts lower atherosclerotic risk factors in a rat hyperlipidemia model," Experimental Gerontology, vol. 44(6), pp. 434-439, 2009.

[43] F. Robbesyn, R. Salvayre and A. Negre-Salvayre, "Dual role of oxidized LDL on the NF-kappaB signaling pathway," Free Radical Research, vol. 38(6), pp. 541-551, 2004.

[44] T. K. Motawi, M. A. Hamed, M. H. Shabana, R. M. Hashem and A. F. Aboul Naser, "Zingiber officinale acts as a nutraceutical agent against liver fibrosis," Nutr Metab (Lond), vol. 8, p. 40, 2011.

[45] F. Peng, Q. Tao, X. Wu, H. Dou, S. Spencer, C. Mang, L. Xu, L. Sun, Y. Zhao and H. Li, "Cytotoxic, cytoprotective and antioxidant effects of isolated phenolic compounds from fresh ginger," Fitoterapia, vol. 83(3), pp. 568-585, 2012.

[46] B. Fuhrman, M. Rosenblat, T. Hayek, R. Coleman and M. Aviram, "Ginger extract consumption reduces plasma cholesterol, inhibits LDL oxidation and attenuates development of atherosclerosis in atherosclerotic, apolipoprotein E-deficient mice," The Journal of Nutrition, vol. 130(5), pp. 1124-1131, 2000.

DOI:10.5963/BER0302001