ผลปกป้องของเคอร์คิวมินอยด์จากขมิ้นชันต่อปฏิกิริยาออกซิเดชันในไลโปโปรตีนชนิดความหนาแน่นต่ำที่ถูกออกซิไดซ์ด้วยอีมิน

นางสาวประภาพร จันทร์เอียด

ศูนย์วิทยทรัพยากร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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จุฬาลงกรณ์มหาวิทยาลัย

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

THE PROTECTIVE EFFECT OF CURCUMINOIDS FROM *CURCUMA LONGA* L. ON HEMIN INDUCED LOW DENSITY LIPOPROTEIN OXIDATION

Miss Prapaporn Chaniad

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Pharmacology
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ประภาพร จันทร์เอียด : ผลปกป้องของเคอร์คิวมินอยด์จากขมิ้นชันต่อปฏิกิริยาออกซิเดชันในไลโปโปรตีนชนิดความหนาแน่นต่ำที่ถูกออกซิไดซ์ด้วยฮีมิน. (THE PROTECTIVE EFFECT OF CURCUMINOIDS FROM *CURCUMA LONGA* L. ON HEMIN INDUCED LOW DENSITY LIPOPROTEIN OXIDATION) อ. ที่ปรึกษา : ผศ. ดร. รัตยา ลือชาพุฒิพร, 113 หน้า.

การเปลี่ยนแปลงของไลโปโปรตีนชนิดความหนาแน่นต่ำ (LDL) โดยปฏิกิริยาออกซิเดชัน มีบทบาทสำคัญต่อพยาธิกำเนิดของการแข็งตัวของหลอดเลือด ฮีมินเป็นสารที่เกิดขึ้นจากการสลายตัวของฮีโมโกลบิน จัดเป็นสารออกซิไดซ์ที่พบว่ามีปริมาณมากขึ้นในพยาธิสภาพของโรคบางโรค เช่น hemoglobinopathies, sickle cell anemia และ thalassemia เหง้าของขมิ้นชันประกอบด้วยสารที่มีฤทธิ์ทางชีวภาพ ได้แก่สารในกลุ่มเคอร์คิวมินอยด์ ซึ่งประกอบด้วย curcumin, demethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC) สารเหล่านี้มีคุณสมบัติเป็นสารต้านออกซิเดชันและสารต้านการอักเสบ การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาและเปรียบเทียบผลการใช้ curcuminoids, curcumin, DMC, BDMC และ Tetrahydrocurcumin (THC) ในการยับยั้งการเกิดปฏิกิริยาออกซิเดชันของ LDL ที่ถูกออกซิไดซ์ด้วยฮีมิน การศึกษานี้ทดลองโดยเติมสารทดสอบที่ความเข้มข้นต่างๆ ลงใน LDL ก่อนเป็นเวลา 30 นาที โดยใช้ α-tocopherol เป็น positive control แล้วจึงเติมฮีมิน 2.5 ไมโครโมลาร์ต่อ LDL 150 ไมโครกรัมโปรตีน/มิลลิลิตร เหนี่ยวนำให้เกิดปฏิกิริยาออกซิเดชันของ LDL เป็นเวลา 24 ชั่วโมง ผลการทดลองพบว่า LDL ที่ถูกออกซิไดซ์ด้วยฮีมินมีระดับ α-tocopherol ลดลง ระดับ thiobarbituric acid reactive substances (TBARs) เพิ่มขึ้นและส่งผลต่อการเปลี่ยนแปลงองค์ประกอบของไขมัน มีผลให้ระดับ cholesteryl arachidonate (CA) และ cholesteryl linoleate (CL) ลดลง 28.88% และ 24.24% ตามลำดับ รวมทั้งอัตราส่วน CL/CO ลดลง

พบว่าสารทดสอบทุกชนิดมีคุณสมบัติเป็นสารต้านออกซิเดชันสามารถปกป้องการเกิดปฏิกิริยาออกซิเดชันของไขมันได้ โดยมีผลยับยั้งการเพิ่มระดับ TBARs การลดลงของระดับ α-tocopherol มีผลปกป้องการลดลงของระดับ CA และ CL รวมทั้งมีผลปกป้องการลดลงของอัตราส่วน CL/CO ในลักษณะที่ขึ้นกับความเข้มข้น โดยความเข้มข้นที่สามารถยับยั้งการลดลงของ CA ได้ 50% (IC_{50}) สำหรับ THC, curcumin, curcuminoids, DMC, BDMC และ α-tocopherol คือ 4.5, 9.3, 10.4, 25.5, 47.3 และ 65.9 μM IC_{50} ในการยับยั้งการลดลงของ CL คือ 5.5, 6.0, 10.8, 16.5, 30.0 และ 60.4 μM ตามลำดับ นอกจากนี้เมื่อเปรียบเทียบฤทธิ์ของสารทดสอบขนาด 10 μM พบว่าสามารถยับยั้งอัตราการเพิ่มขึ้นของระดับ TBARs และอัตราการลดลงของระดับ α-tocopherol ได้ โดย THC, curcumin และ curcuminoids มีผลยับยั้งได้ใกล้เคียงกันและมากกว่า DMC > BDMC > α- tocopherol ลำดับความสามารถในการปกป้องการเกิดปฏิกิริยาออกซิเดชันของ LDL ที่ถูกเหนี่ยวนำด้วยฮีมินคือ THC ≥ curcumin ≥ curcuminoids > DMC > BDMC > α- tocopherol ($p<0.05$)

สาขาวิชา เภสัชวิทยา ลายมือชื่อนิสิต.....

ปีการศึกษา 2550 ลายมือชื่ออาจารย์ที่ปรึกษา.....

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KEY WORD: HEMIN / CURCUMINOIDS / OXIDIZED LDL / CHOLESTERYL ESTER / TETRAHYDROCURCUMIN

PRAPAPORN CHANIAD: THE PROTECTIVE EFFECT OF CURCUMINOIDS FROM *CURCUMA LONGA* L. ON HEMIN INDUCED LOW DENSITY LIPOPROTEIN. THESIS ADVISOR: ASST. PROF. RATAYA LUECHAPUDIPORN, 113 pp.

Oxidative modification of low-density lipoprotein (LDL) plays a major role in the pathogenesis of atherosclerosis. Hemin, a degradation product of hemoglobin, is one of oxidative mediator elevated in pathological cases like severe hemoglobinopathies, sickle cell anemia and thalassemia. The rhizome of *Curcuma longa* L. contain bioactive substances referred to as curcuminoids, including curcumin, demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC), and these molecules have antioxidant and anti-inflammatory activities. The objective of this study is to compare the protective effect of curcuminoids, curcumin, DMC, BDMC and tetrahydrocurcumin (THC) on hemin induced LDL oxidation (he-oxLDL).

The pre-incubation of LDL with various concentrations of the tested compounds were performed; and then oxidized by 2.5 μM hemin/150 μg LDL protein. α-Tocopherol was used as a positive control. He-oxLDL at 24 hour-oxidation was shown by the depletion of α- tocopherol, the increase of the thiobarbituric acid reactive substances (TBARs) level and the alteration of lipid composition in LDL. The levels of cholesteryl arachidonate (CA) and cholesteryl linoleate (CL) were decreased by 28.88% and 24.24% ($p<0.05$) respectively, including the CL/CO ratio was also decreased.

All tested compounds possess an antioxidant activity which protects hemin-induced lipid peroxidation by inhibition of TBARs formation, the depletion of α-tocopherol, the decrease of lipid (CA and CL) and decreased of CL/CO ratio in a concentration dependent manner. The 50% inhibition concentration (IC_{50}) of the damage of CA were 4.5, 9.3, 10.4, 25.5, 47.3, and 65.9 μM and those of CL were 5.5, 6.0, 10.8, 16.5, 30.0 and 60.4 μM for THC, curcumin, curcuminoids, DMC, BDMC and α-tocopherol, respectively. In addition, when compare the effect of tested compounds 10 μM found that these tested compounds were able to inhibit rate of TBARs formation and decreasing rate of α-tocopherol. THC has a comparable effect with curcumin and curcuminoids to inhibit these rate and more than DMC, BDMC and α- tocopherol respectively. We conclude that a significant protective effect on he-oxLDL is in the order of THC ≥ curcumin ≥ curcuminoids > DMC > BDMC > α- tocopherol ($p<0.05$).

Field of study Pharmacology Student's signature Prapaporn Chaniad

Academic year 2007 Advisor's signature Rataya L

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LIST OF ABBREVIATIONS

α-tocopherol	Alpha-tocopherol
Apo	Apoprotein
BHT	Butylated hydroxytoluene
BDMC	Bisdemethoxycurcumin
β-thal/HbE	β-thalassemia/hemoglobin E
CA	Cholesteryl arachidonate
CE	Cholestery ester
CL	Cholesteryl linoleate
CO	Cholesteryl oleate
Conc	Concentration
CP	Cholesteryl palmitate
°C	Degree celcius
DMC	Demethoxycurcumin
FC	Free cholesterol
G	Gram
HDL	High density lipoprotein
HPLC	High perfomance liquid chromatography
hr	Hour
he-oxLDL	Hemin oxidized Low Density Lipoprotein
H_2O_2	Hydrogen peroxide
LDL	Low Density Lipoprotein
MDA	Malondialdehyde
mg	Milligram
min	Minute
ml	Milliliter
mM	Millimolar
mm	Millimeter
μg	Microgram
μl	Microliter
μM	Micromolar
NA_2EDTA	Ethylenediamine tetraacetic acid disodium salt

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ND	Not determine
nLDL	Native Low Density Lipoprotein
nm	Nanometer
nmol	Nanomole
OD	Optical density
ox-LDL	Oxidized Low Density Lipoprotein
PBS	Phosphate buffer saline
PUFA	Polyunsaturated fatty acid
rpm	Revolution per minute
S.E.M.	Standard error of mean
TBARs	Thiobarbituric acid reactive substances
TC	Total cholesterol
THC	Tetrahydrocurcumin

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CHAPTER I

INTRODUCTION

1. Background and rationale

Oxidative modification of low density lipoprotein (LDL) plays an important role in the initiation and progression of atherosclerosis (1, 2). The atherogenesis related vascular complications such as pulmonary thromboembolism, cerebral thrombosis and chronic leg ulcers are commonly found in β-thalassemia patients (3). β-Thal/HbE patients are in the oxidative stress condition which cause lipid peroxidation in blood circulation especially in LDL. In addition, using the technique of electron spin resonance (ESR) spectroscopy, high level of hemin found in the serum of β-thal/HbE but undetectable in normal serum. It was suggested that serum hemin readily catalyzed free radical reactions and it may be a major pro-oxidant in blood circulation of β-thal/HbE (4).

Hemin is a degradation product of hemoglobin that may drive from hemoglobin in circulating erythrocytes. Hemoglobin and free hemin appear in plasma following intravascular hemolysis. Increased hemin is associated with many pathological stages, like severe hemoglobinopathies, sickle cell anemia and thalassemia (2, 5). The kinetics of hemin distribution in plasma shown that as much as 80% of total hemin bound initially to LDL and HDL, the plasma components which are most susceptible to oxidation. LDL binds hemin faster than any other proteins (HDL, albumin and hemopexin) (6). It was suggested that the hemin-LDL complex in plasma may exist and its oxidative potential should be considered pro-atherogenic (7).

The study of Luechapudiporn (8) have found that the oxidative modification of lipoprotein in β-thal/HbE patients are shown by the increase of lipid peroxidation (thiobarbituric acid reactive substances; TBARs), the depletion of α-tocopherol and the decrease of cholesteryl linoleate (CL) level as well as the ratio of CL to cholesteryl oleate (CO).

For *in vitro* study, hemin is a powerful inducer of LDL oxidation. During incubation of LDL with hemin, it greatly enhanced the oxidation of LDL and led to large increases in the formation of TBARs, lipid hydroperoxides and lipid conjugated dienes, while lipoprotein polyunsaturated fatty acyl-containing cholesteryl esters

content were decreased. The exposure of LDL to hemin in the presence of H_2O_2 catalyzed the rapid peroxidation of LDL to substances highly cytotoxic for endothelial cells, it may also promote foam cell production and the formation of the early fatty streak lesions of atherosclerosis (9,10).

The data from *in vitro* study of LDL oxidation induced by hemin have specific characteristic similar to LDL in β-thal/HbE patients which have oxidative stress condition. Until present, there are many attempt to use antioxidant in β-thal/HbE patients to escape the suffering from oxidative stress. Thereby use *in vitro* model is appropriate to study the antioxidant effect of test compounds on LDL oxidation.

Curcuminoids is one of the powerful antioxidant, refer to a group of phenolic compounds as a yellow pigment present in the rhizome of the herb *Curcuma longa* L. (turmeric). Three curcuminoids were isolated from turmeric composed of curcumin, demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC) (11-13).

Curcumin has been shown to possess a potent antioxidant comparable to α-tocopherol. Antioxidant activities of curcuminoids have been studied in several models. *In vitro* study, curcumin has been shown to be a potent scavenger of a variety of reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals and nitrogen dioxide radicals (14,15). These ROS are important to initiate lipid peroxidation and have a main role in the inflamation, in heart diseases and in cancer (16). In rat, curcumin has been shown to decrease activity of xanthine oxidase, reactive oxygen species and lipid peroxides while increase the level of superoxide dismutase, catalase, glutathione peroxidase and glutathione-s-tranferase (17). One clinical study about the administration of curcuminoid extract capsules in β-thal/HbE patients found that curcuminoids have an antioxidant effect and improved all biochemical parameters of oxidative stress in patients (18). Antioxidant activities of DMC and BDMC have been reported in the model system of AAPH induced RBC hemolysis, DPPH radical scarvenging and AAPH induced oxygen uptake in LDL. In addition, antioxidant activities of THC have already been studied both *in vitro* and *in vivo*.

We have previously shown that curcumin can protect the LDL oxidation induced by hemin and it was able to protect LDL oxidation more than α-tocopherol approximately 2-3 times (19). However, to date there has been no comparative study on the protective effect of curcumin with its derivative and its metabilite on hemin

induced LDL oxidation (he-oxLDL). Therefore, the aim of this study is to compare the protective effect of curcuminoids, curcumin, DMC, BDMC and THC, one of the major metabolites of curcumin on he-oxLDL.

2. Objective

To compare the protective effect of curcuminoids, curcumin, demethoxycurcumin, bisdemethoxycurcumin and tetrahydrocurcumin on hemin induced LDL oxidation (he-oxLDL).

3. Hypothesis

Curcuminoids and its derivatives are able to protect LDL oxidation induced by hemin.

4. Expected benefit and application

Knowledge from this studies lead to obtain information about comparative potency of curcuminoids and its derivatives on antioxidant effect for protect LDL oxidation induced by hemin.

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CHAPTER II

LITERATURE REVIEW

1. Low Density Lipoprotein

Low density lipoproteins (LDL) is one class of lipoproteins which are submicroscopic particles composed of lipid and proteins held together by non covalent forces. LDL is the major lipid carrier in plasma (20), its derived from very low density lipoproteins (VLDL) which is synthesized in the liver and is converted by the action of lipoprotein lipase, situated on the blood capillary walls of adipose tissue, muscle and other ogans to form intermediate density lipoproteins. A large part of these, about half in the case of humans, is normally processed further to form LDL. The conversion of VLDL into LDL results in the loss of triglyceride, phospholipids and apolipoproteins other than apo B 100 which remains as the major apolipoprotein associated with the resulting LDL particles. LDL is cleared from the plasma by a regulated pathway involving endocytosis following binding to apo B, E receptors present in the liver and other tissues (21).

LDL molecules are large, spherical particles with a diameter of 225-275°A, a mean molecular weight of 2.5 million (figure 2.1). LDL which can be isolated by ultracentrifugation within a density range of 1.019 to 1.063 g/mL. Each LDL particle would contain about 1600 molecules of cholesteryl ester (CE), and 170 molecules of triglycerides (TG), which form a central lipophilic core. Cholesteryl esters (CEs) are most abundant in core of LDL. The structure of CE consist part of cholesterol and part of fatty acid such as arachidonic acid, linoleic acid, oleic acid and palmitic acid, linking with ester bound (figure 2.2) (22).

The core is surrounded by a monolayer of about 700 phospholipid molecules and 600 molecules of free cholesterol. The polar heads of the phospholipids are located at the surface of the LDL particle and contribute to the solubility of LDL in an aqueous phase. Embedded in the outer layer is a large protein termed apolipoprotein B (apo B). The apo B is an exceptionally large protein consisting of 4536 amino acids (23).

Moreover, LDL has a number of lipophilic antioxidants which protect the PUFAs from free radical attack and oxidation. The major antioxidant is α-tocopherol; a LDL paricle holds about 6 molecules of α-tocopherol. All other antioxidants (γ-tocopherol, carotenoids, oxycarotenoids and ubiquinol-10) are present in much smaller amounts: for instance, only about one third of the LDL molecules contain beta-carotene. It has been proposed that α-tocopherol is the only important antioxidant, whereas carotenoids and ubiquinol-10 play only a very minor role in protecting LDL from oxidation (22).

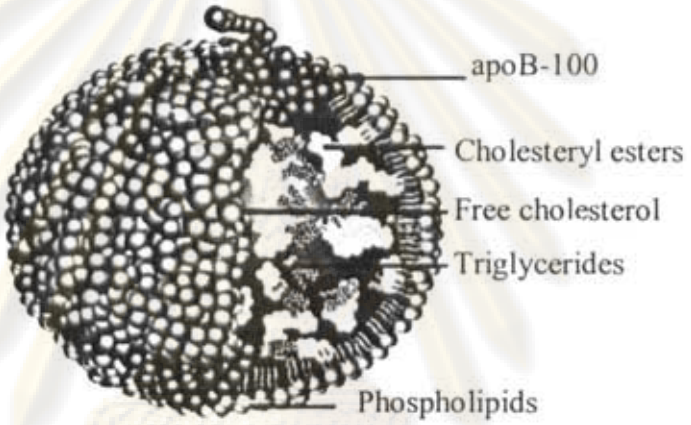

Figure 2.1 The structure of LDL (24)

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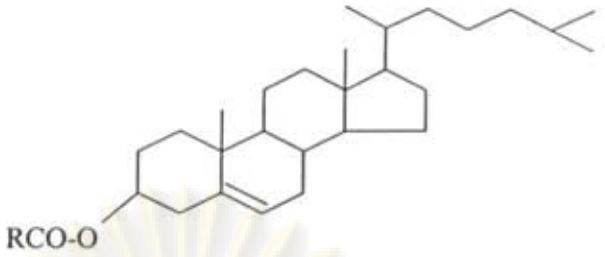

Cholesteryl ester

R represents part of fatty acid:

Arachidonic acid (C20:4)

Linoleic acid (C18:2)

Oleic acid (C18:1)

Palmitic acid (C16:0)

Figure 2.2 The chemical structures of cholesteryl ester and fatty acid

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2. Oxidized Low density lipoprotein

Oxidation of LDL is a lipid peroxidation process in which the PUFAs contained in the phospholipids are transformed into lipidhydroperoxides and then to some unsaturated aldehydes. These products can neutralize some positive charges from lysine, arginine and histidine residues and increase the apo B affinity to the scavenger receptor.

Endothelial cells, smooth muscle cells and macrophages have all been shown to be capable of oxidizing LDL *in vitro*. LDL can also be oxidized in a cell-free medium with a sufficiently high concentration of copper and iron. The hypothesis of the mechanism of copper stimulated oxidation of LDL is the following: traces of transition metal ions (copper or iron) can decompose small amounts of preformed lipid hydroperoxides (LOOH) contained in the LDL, in reactive alkoxy ($LO^{\cdot}$) and peroxy radicals ($LOO^{\cdot}$). These radicals begin the lipid peroxidation chain reaction in which the PUFA of the LDL (mainly linoleic and arachidonic acid) are oxidized to lipid hydroperoxides (22). During LDL oxidation, both the lipids and apo B present in LDL are modified. Reactive oxygen species induce fragmentation of apo B to produce peptides ranging from 14-500 kDa. The PUFA in CEs, phospholipids, and TGs are also subjected to free radical-initiated oxidation to yield a broad array of smaller fragments. The fragments further participate in chain reactions that propagate and amplify the damage (80). Before being completely oxidized, LDL particles have to undergo some modifications. At first, native LDL particles contain one intact polypeptide (apoB-100), no lipidperoxides or aldehydes, and are enriched in PUFA and antioxidants. Minimally modified oxidized low-density lipoprotein (MM-LDL) particles are characterized by oxidation of phospholipids on the surface of LDL particles. At this stage, the apo B moiety of LDL is intact but the particle has lost PUFA and antioxidant compounds. Since the structure of the apo B molecule is unchanged, LDL particles are not yet recognized by scavenger receptors of macrophages, although they are still recognized by LDL receptors. Fully ox-LDL particles are recognized by scavenger receptors of macrophages and are internalized (81).

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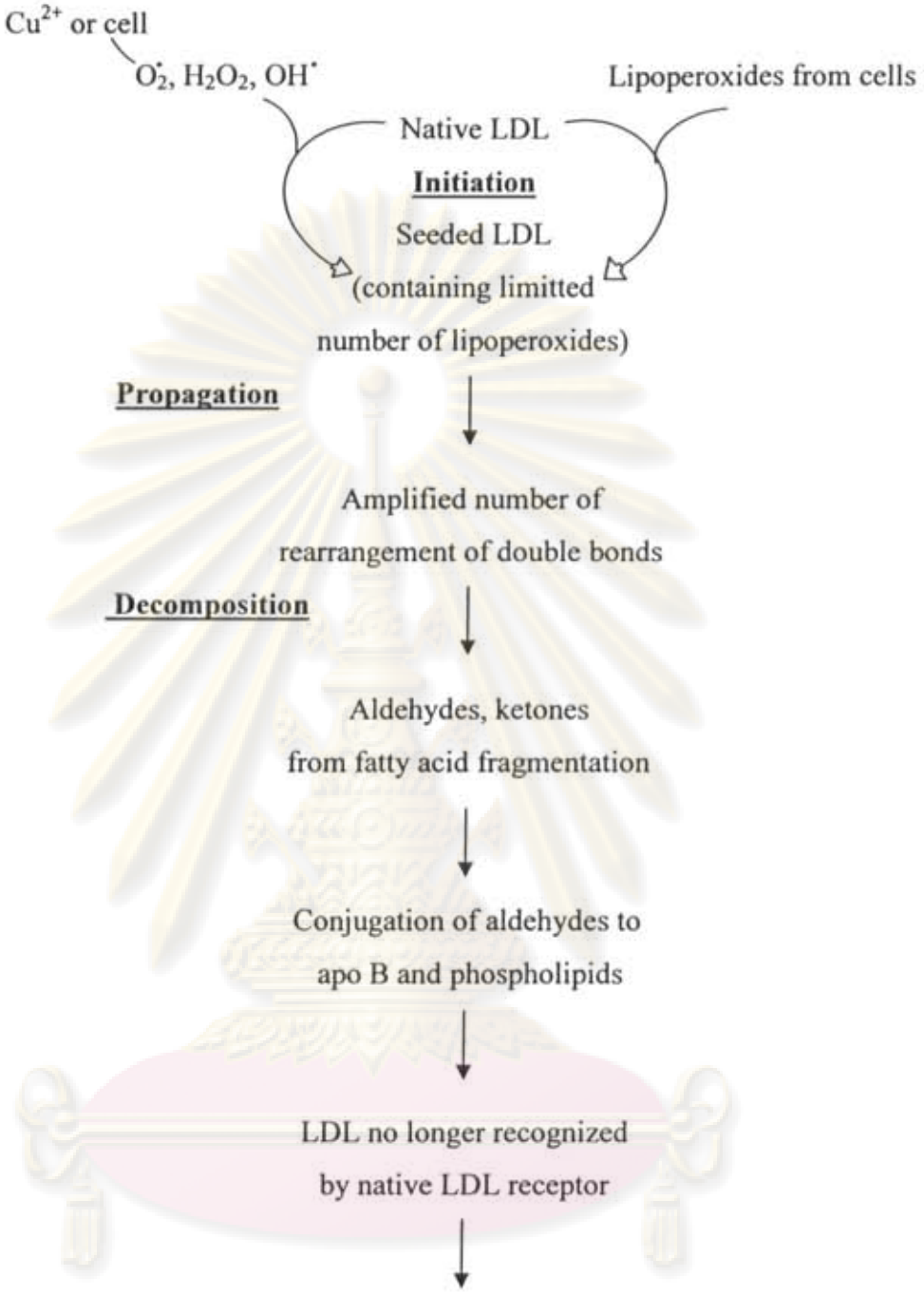

Figure 2.3 Mechanism of LDL oxidation (25)

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Biological effects and in vivo existence of oxidized low density lipoprotein.

Ox-LDL exerts biological effects that contribute to the evolution of the atherosclerosis lesion. During oxidation minimally modified LDL (MM-LDL) is intially formed in the subendothelial space. MM-LDL is typified by mild lipid peroxidation and uptake by the classical LDL receptor, it can induce monocyte-endothelial adhesion and secretion of monocyte chemotactic protein-1 (MCP-1) and macrophage colony stimulating factor (M-CSF) from endothelium. This results in monocyte binding to the endothelium and subsequent migration to the subendothelial space via MCP-1, where M-CSF promotes their differentiation into macrophages. Macrophages in turn oxidize MM-LDL into a more oxidized form.

Ox-LDL is no longer recognized by the LDL receptor but is taken up by the scavenger receptor on the macrophages, resulting in foam cell formation. It is a potent chemoattractant for monocytes and inhibits macrophage motility, thereby promoting retention of macrophages in the arterial wall. Ox-LDL is cytotoxic and promotes endothelial dysfunction and evolution of the fatty streak into a more advanced lesion: it can also promote atherogenesis by altering expression of genes in the arterial wall. Ox-LDL can adversely affect coagulation by including tissue factor and plasminogen activator inhibitor-1 synthesis. Products of ox-LDL have been shown to impair gene expression of tomor necrosis factor and platelet-derived growth factor. Ox-LDL inhibits endothelium-dependent vasodilation by inhibiting endothelium-derived relaxation factor (EDRF). Another atherogenic property of ox-LDL is its immunogenicity. Malondialdehyde-modified LDL has been shown to stimulate formation of autoantibodies and immune complexes of LDL aggregates that are efficiently internalized by macrophages by Fc receptos, thus promoting further cholesterol accumulation.

In addition to M-CSF and MCP-1, ox-LDL stimulates interleukin-1 (IL-1) release from marophages. IL-1 has been shown to induce smooth muscle cell proliferation and monocyte-endothelial cell adhesion. Also, IL-1b mRAN has been found to be a higher levels in atherosclerotic lesions (26).

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Table 2.1 Potential mechanisms by which ox-LDL may be atherogenic (25).

- It has enhanced uptake by macrophages leading to cholesteryl ester enrichment.
- It is chemotactic for circulating monocytes.
- It inhibits the mobility of tissue macrophages.
- It is cytotoxic.
- It can alter gene expression of neighboring cells such as induction of MCP-1 and colony- stimulating factors.
- It is immunogenic and can elicite autoantibody formation.
- It can adversely alter coagulation pathways.
- It can adversely alter vasomotor properties of coronary ateries.

Inhibition of oxidation of LDL

Areobic organism are protected against oxidative damage by an array of defense systems. Among others, the radical scavenging antioxidants inhibit chain initiation and/or break chain propagation. Vitamin C, uric acid and bilirubin act as hydrophilic antioxidants, while vitamin E, ubiquinol and carotenoids act as lipopphilic antioxidants. The relative contribution of these antioxidants depends very much on physical factors as well as their inherent, chemical reactivities toward free radicals.

When aqueous peroxyl radicals are formed in the whole blood or plasma, vitamin C acts as the primary defense, being consumed most rapidly. However, two points must considered. Firstly, the activity of the attacking radical is important. The more active the attacking radicals becomes, the less selective it is, and in such case the concentration of the antioxidant may become more important than the reactivity toward the radical. Hence, uric acid may be more effective than vitamin C against hydroxyl radical formed in the plasma. Secondly, the location of free radicals and antioxidant effects the reaction. It has been found, for instance, that neither vitamin C nor uric acid can scavenge lipophilic radicals within LDL efficiently. Its was also found that the addition of plasma did not suppress the oxidation of meyhyl linoleate micelles induced by lipophilic radicals, although it acted as a potent antioxidant against the oxidation induced by aqueous radicals. These serults suggest that water-soluble radicals scavenging antioxidants can inhibit the chain initiation by scavenging

aqueous radicals, but that they can not break the chain propagation taking place within lipophilic LDL. This also means the lipophilic antioxidants are required in LDL to effectively suppress its oxidative modification.

Vitamin E, ubiquinol, and carotenoids are present in LDL. Although the amount of ubiquinol (abount only 0.1 molecule per LDL particle) is much smaller than that of vitamin E (6.4 molecules α-tocopherol and 0.5 molecules γ-tocopherol per molecules of LDL) and the chemical reactivity of ubiquinol toward peroxyl radicals is smaller than that of α-tocopherol, it has been found that ubiquinol is consumed faster than vitamin E during the oxidation of LDL. This may arise from the direct scavenging of radicals, reduction of vitamin E radical and/or autoxidation of ubiquinol. However, vitamin C can reduce vitamin E radical in LDL. Carotenoids are weak radical-scavenging antioxidants and they are consumed after most of the ubiquinol and vitamin E is depleted. Vitamin E is by far the most abundant and reactivity antioxidant in LDL.

Probucal is known as a drug which prevents atherogenesis by acting as an antioxidant and suppressing the oxidative modification of LDL, in addition to its recognized effects of lowering cholesterol level. The chemical reactivity of probucal toward peroxyl radical is much amaller than that of α-tocopherol. The antioxidant activity against LDL oxidation is determined by physical factors as well as by the chemical reactivity of antioxidant (27).

Lipid peroxidation

Lipid peroxidation is initiated by free radical attack on a double bond associated with a PUFA. This results in the removal of a hydrogen atom from a methylene (CH_2) group, the rate of which determines the rate of initiation, a key step. Molecular rearrangement of the resulting unstable carbon radical results in a more stable configuration, a conjugated diene. The conjugated diene reacts very quickly with molecular oxygen, and the peroxyl radical thus formed is a crucial intermediate. A PUFA peroxyl radical in LDL may abstract a hydrogen atom from an adjacent PUFA to form a hydroperoxide and another lipid radical, a reaction which results in chain propagation. Removal of hydrogen atoms by the peroxyl radical from other lipids, including cholesterol, eventually yields oxysterols. Lipid hydroperoxides fragment to shorter-chain aldehydes, including malondialdehyde and 4

hydroxynonenal. These reactive aldehydes in turn may bind to ε-amino groups of apo B-100, giving the protein an increased net negative charge. The classical LDL receptor recognizes a specific domain of positive charges from lysine, arginine and histidine residues on apo B. Alteration of this domain results in failure of binding by the apo B/E receptor, and an increase in negative surface charge on apo B-100 results in increased recognition by the scavenger receptor. In the presence of a lipid phase chainbreaking antioxidant such as α-tocopherol, the peroxyl radical may be scavenged. The tocopheroxyl radical thus formed has very low reactivity and will generally result in chain termination (28).

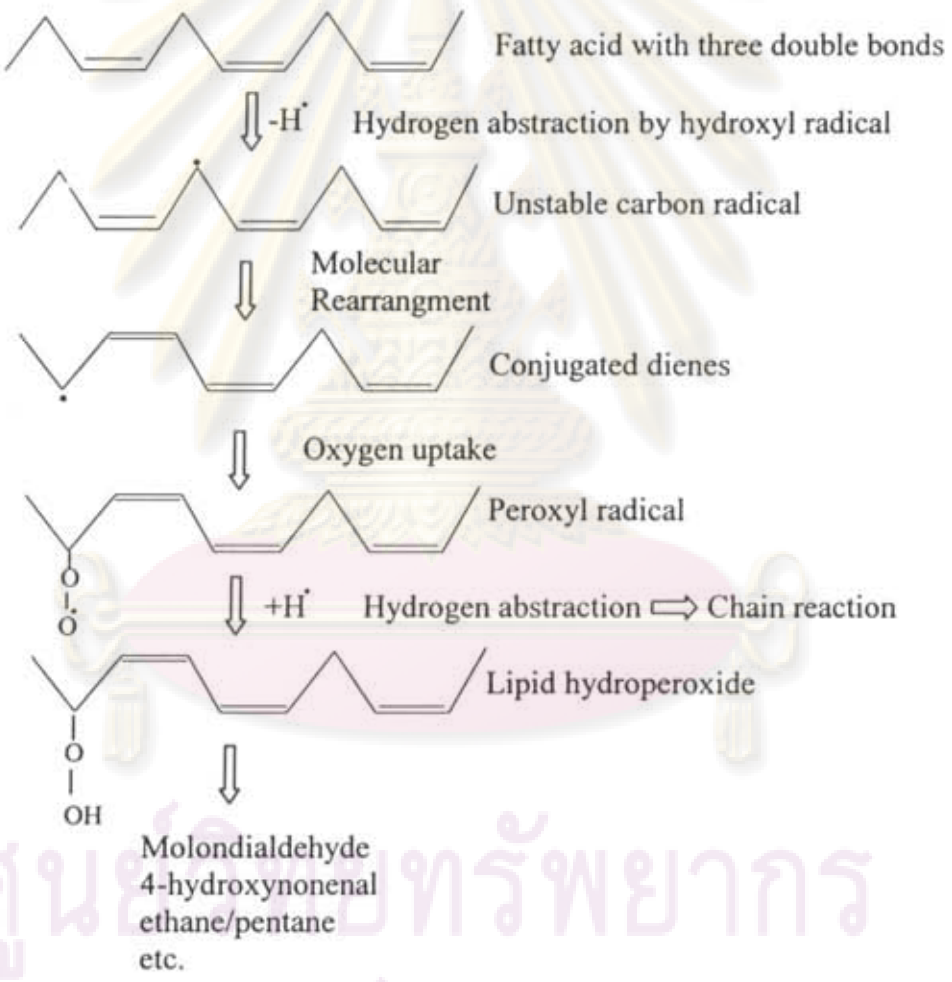

Figure 2.4 Basic reaction sequence of lipid peroxidation

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3. Hemin

The amphipatic hemin is a protoporphyrin IX with a coordinated Fe^{3+}, formed in the *in vivo* breakdown of heme (protoporphyrin IX- Fe^{2+}), that is usually bound to hemoglobin and myoglobin (7). Hemoglobin and free hemin appear in plasma following intravascular hemolysis (2). Erythrocytes lysis and hemin overload result in the accumulation of hemin in blood plasma, with further import to various organs and tissues (9). Hemin is a potent hemolytic agent (29) it was found to be elevated in pathological case like severe hemoglobinopathies, sickle cell anemia and thalassemia. Increased hemin is associated with many pathological stages (2, 5). In the study of Miller and Shaklai (6) showed that first seconds after hemin appearance in plasma, more than 80% of this powerful oxidizer binds to lipoproteins (LDL and HDL), LDL binds hemin faster than any other proteins and only the remaining 20% binds to antioxidative human serum albumin (HSA) and hemopexin (HPX), that bind hemin most strongly. While it may be that HPX binds hemin somewhat more strongly than does HSA, the large ratio of concentrations of albumin to hemopexin (100 : 1) in all species examined, should make albumin the main carrier of hemin (5).

The half time of the hemin-LDL complex in plasma, initially comprising 27% of total hemin, was more than 20 seconds (6).

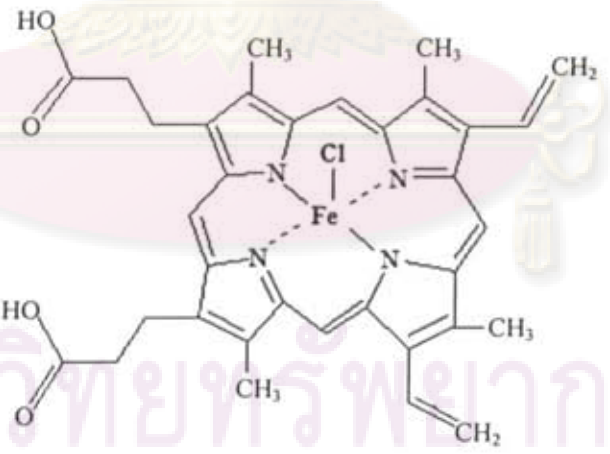

Figure 2.5 The chemical structure of hemin

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In vivo, hemin may become available in blood plasma to participate in LDL oxidation as a result of hemoglobin oxidation and decomposition. During intravascular hemolysis, free hemoglobin released into blood plasma is rapidly oxidized to methemoglobin and subsequently, dissociates into ferriheme (i.e. hemin) and globin (10).

Several researchers have pointed out that hemin is an effective *in vitro* inducer of LDL oxidation. Incorporation of hemin into the LDL particle results in delivery of pro-oxidant Fe^{3+} to the hydrophobic core of the lipoprotein where it initiates lipid peroxidation. The hemin oxidation, producing a variety of LDL lipid oxidation products and apo B crosslinking, resulting in transformation of the lipoprotein particle to a pro-atherogenic form that both is cytotoxic to aortic endothelial cells (9) and accumulates within monocyte-derived macrophages to generate the lipid-laden foam cell characteristic of the early atherosclerotic lesion (7).

The toxic effects of hemin on erythrocytes include the induction of potassium leakage (29) the dissociation of erythrocyte membrane skeletal proteins (30) and inhibition of a number of erythrocyte enzymes (31).

4. α-Tocopherol

α-Tocopherol is the most active form of vitamin E and the most abundant lipid soluble antioxidant in human LDL. The principle role of α-tocopherol as antioxidants is to destroy free radicals which could initiate a chain reaction, particularly in highly unsaturated lipids. α-Tocopherol to suppress lipid peroxidation is generally attributed to its scavenging of chain-carrying lipid peroxyl radicals and being converted to α-tocopheryl radicals. α-Tocopheroxyl radicals is poorly reactive compared with most other radicals and rapidly degrades to other products. Moreover, α-tocopheroxyl radicals were reduced by ascorbic acid to the antioxidant tocopherol. The antioxidant mechanism of α-tocopherol cooperated with several antioxidant such as vitamin C and glutathione (32).

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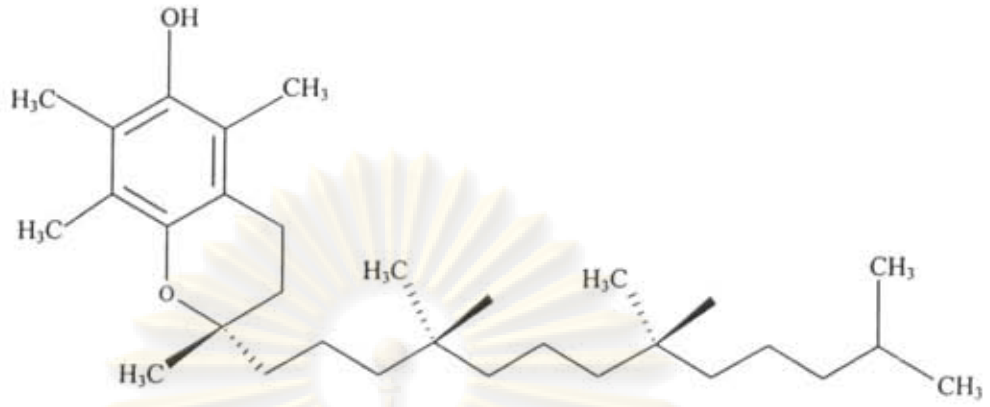

Figure 2.6 The chemical structure of α-tocopherol

5. Curcuminoids and its derivatives

Curcuminoids refer to a group of phenolic compounds as a yellow pigment present in the rhizome of the herb *Curcuma longa* L. (turmeric), which is commonly used as a spice and food colorant (12, 13).

Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Liliopsida
Subclass	Zingiberidae
Order	Zingiberales
Family	Zingiberaceae
Genus	Curcuma L.
Species	*Curcuma longa* L.

There are several reports indicating a variety of biological and pharmacological activities of turmeric such as anti-inflammatory, anticarcinogenic, antimicrobial, antiparasitic, antimutagenic, anticancer and antioxidant. Three curcuminoids were isolated from turmeric compose of curcumin [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], demethoxycurcumin (DMC) [1-(4-hydroxyphenyl)-7-(hydroxy-3-methoxyphenyl-1,6-heptadiene-3,5-dione] and bisdemethoxycurcumin (BDMC) [1,7-bis-(4-hydroxyphenyl)-1,6-heptadiene-3,5-

dione] (Figure 2.6). A purified extract containing a mixture of the three curcuminoids i.e. curcumin (75–81%), DMC (15–19%) and BDMC (2.2–6.6%) (13, 14).

Chemical properties

Curcumin (diferuloylmethane) is a bis-, β-unsaturated β-diketone. Curcumin exists in equilibrium with its enol tautomer. The bis-keto form predominates in acidic and neutral aqueous solutions and in the cell membrane. At pH 3–7, curcumin acts as an extraordinarily potent H-atom donor. This is because, in the keto form of curcumin, the heptadienone linkage between the two methoxyphenol rings contains a highly activated carbon atom, and the C–H carbon bonds on this carbon are very weak due to delocalisation of the unpaired electron on the adjacent oxygens. In contrast, above pH 8, the enolate form of the heptadienone chain predominates and curcumin acts mainly as an electron donor, a mechanism more typical for the scavenging activity of phenolic antioxidants (Figure 2.7) (48).

Curcumin is relatively insoluble in water, but dissolves in acetone, dimethylsulphoxide and ethanol. Curcumin is unstable at basic pH and degrades within 30 min to trans-6-(40-hydroxy-30-methoxyphenyl)-2,4- dioxo-5-hexanal, ferulic acid, feruloylmethane and vanillin (33). Under acidic conditions, the degradation of curcumin is much slower, with less than 20% of total curcumin decomposed at 1 h (34). As a result of light sensitivity, samples containing curcumin should be protected from light. Above pH 7, curcumins hue is less yellow and more red. Curcumin has a molecular weight of 368.37 and a melting point of 183°C, DMC; MW 338 and BDMC; MW 308. (35, 36).

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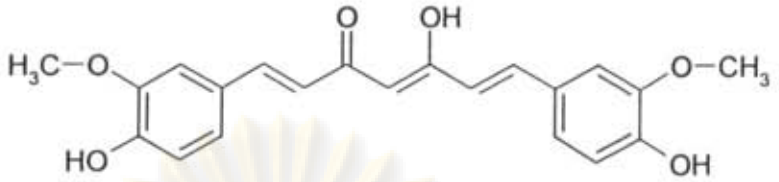
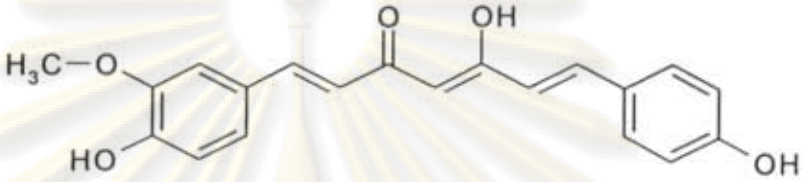
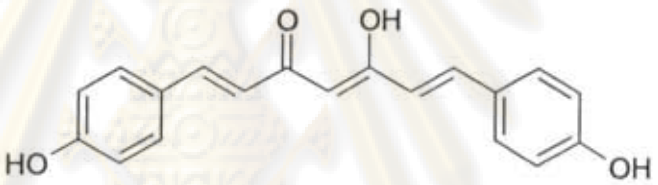


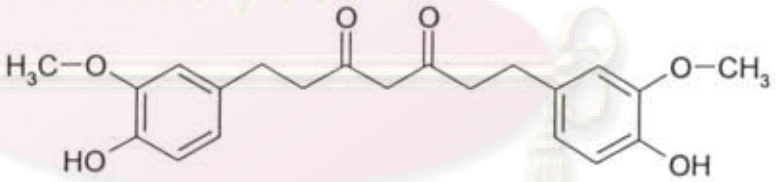
Curcumin



Demethoxycurcumin



Bisdemethoxycurcumin



Tetrahydrocurcumin

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Figure 2.7 The chemical structures of curcumin and its derivatives

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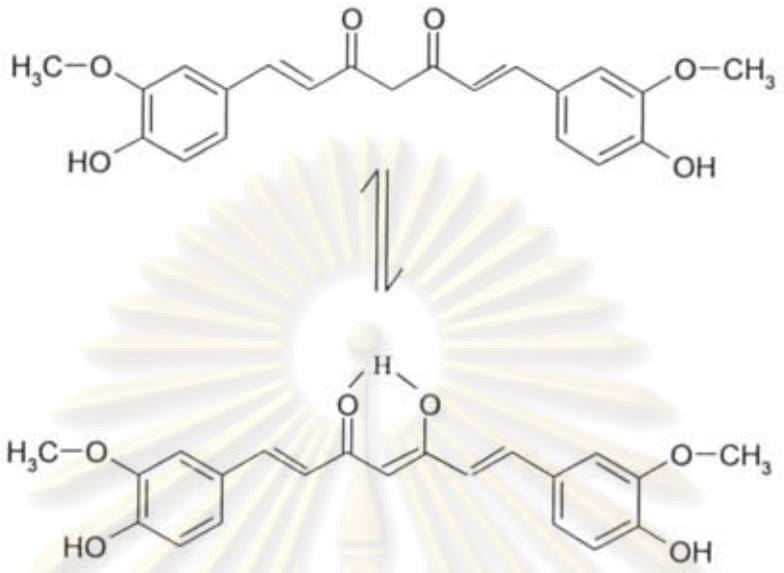

Figure 2.8 Tautomerism of curcumin under physiological conditions. Under acidic and neutral conditions, the bis-keto form (top) predominates, whereas the enolate form (below) is found above pH 8.

Pharmacokinetics and metabolism

The uptake, distribution and excretion of curcumin in Sprague-Dawley rats has been studied. When administered orally in a dose of 1 g/kg, curcumin was excreted in the faeces to about 75%, while negligible amounts of curcumin appeared in the urine. Measurements of blood plasma levels and biliary excretion showed that curcumin was poorly absorbed from the gut (37). A study of oral curcumin administered to rats demonstrated 60% absorption of curcumin and presented evidence for the presence of glucuronide and sulphate conjugates in urine (38). The same investigators proceeded to study the bioavailability of curcumin using ^{3}H-radiolabelling, oral administration resulted in the vast majority of the oral dose being excreted in faeces and only one-third was excreted unchanged (39,40). On the other hand, Holder et al (41) reported that although some curcumin was found in bile after i.v. administration of 50 mg/kg [^{3}H] curcumin in rat, the majority of radioactivity in the bile was present in glucuronides of tetrahydrocurcumin (THC) and hexahydrocurcumin (HHC), which are the hydrogenated metabolites. In the study of the pharmacokinetic properties of curcumin in mice. After intraperitoneal curcumin (0.1 g/kg) to mice, about 2.25 μg/ml of curcumin appeared in the plasma in the first

15 min. One hour after administration are highest follow by spleen, liver and kidneys. The chemical structures of metabolites, determined by mass spectrometry, suggested that curcumin was first biotransformed to dihydrocurcumin and tetrahydrocurcumin and these compounds subsequently were converted to monoglucuronide conjugates. These results together with previous finding, suggest that curcumin-glucoronide, dihydrocurcumin-glucoronide, tetrahydrocurcumin-glucoronide and tetrahydro curcumin are major metabolites of curcumin *in vivo* (42).

Preclinical studies of characterization of metabolites of the chemopreventive agent curcumin in human and rat hepatocytes and in the rat *in vivo* using modern high pressure liquid chromatography (HPLC) techniques demonstrate the major metabolites in suspensions of human or rat hepatocytes were identified as hexahydrocurcumin and hexahydrocurcuminol. In rats, curcumin administered i.v. (40 mg/kg) disappeared from the plasma within 1 h of dosing. After p.o. administration (500 mg/kg) the major products of curcumin biotransformation identified in rat plasma were curcumin glucuronide and curcumin sulfate whereas hexahydrocurcumin, hexahydrocurcuminol and hexahydrocurcumin glucuronide were present in small amounts (43). A study of high dose curcumin (2% in the diet, equating to approximately 1.2 g curcumin per kg body weight) for 14 days has shown that low nanomolar levels are detectable in plasma, with concentrations in liver and colon mucosal tissue ranging from 0.1 to 1.8 nmol/g tissue (44).

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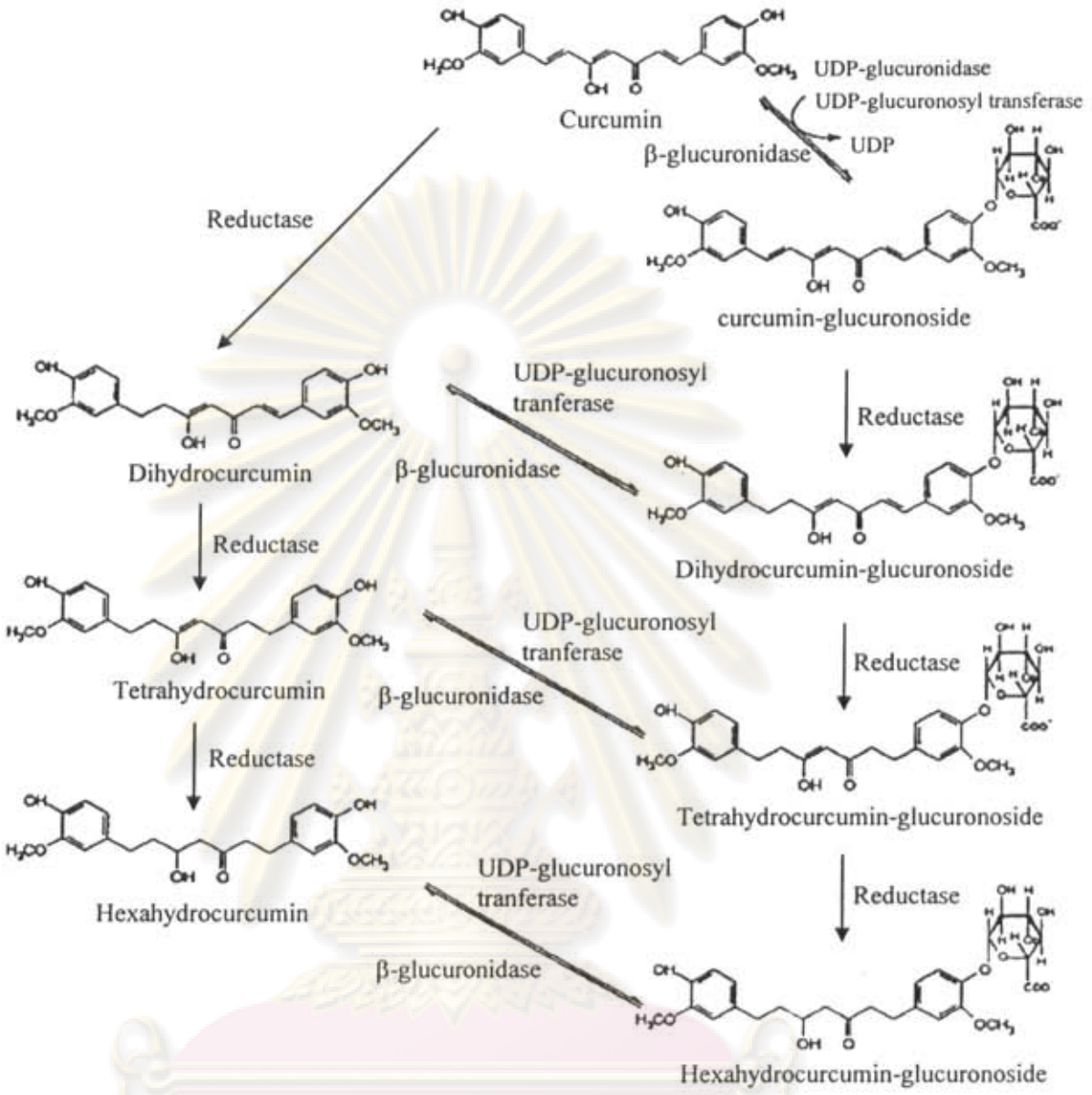

Figure 2.9 Biotransformation of curcumin in mice (42)

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Antioxidant activity

Curcumin, exhibits strong antioxidant activity, comparable to vitamin C and E (45). It was shown to be a potent scavenger of a variety of reactive oxygen species. Pulla Reddy and Lokesh (46) observed that curcumin is capable of scavenging oxygen free radicals such as superoxide anions and hydroxyl radicals, which are the initiators of lipid peroxidation and nitrogen dioxide radicals (15, 16). Curcumin is a good antioxidant and inhibits lipid peroxidation in rat liver microsomes, erythrocyte membranes and brain homogenates (49). Unnikrishnan and Rao (50) studied the antioxidative properties of curcumin on 0.6 mM nitrate-induced methemoglobin formation in hemolysate and intact erythrocytes. Curcumin can protect hemoglobin from oxidation both in hemolysate and intact erythrocytes. In the presence of curcumin, the oxidation process was delayed in a dose-dependent manner. Sreejayan and Rao (36) have reported that three curcuminoids were inhibitors of lipid peroxidation in rat brain homogenates and rat liver microsomes. All of these compounds were more active than tocopherol as reference and curcumin showed the better results. Antioxidant effect of curcumin on human red blood cell and their membrane, curcumin at concentration of 4-100 μM protected RBC against 1.0 mM H_2O_2–induced lysis in a dose dependent manner. Curcumin 100 μM cause significant inhibition of the lipid peroxidation in normal RBC ghost supplemented with exogenous iron and in β-thalassemic ghosts containing endogenous iron deposits (52).

Ahsan et al (14) studied structure activity relationship between curcumin, DMC and BDMC. Curcumin was found to be the most effective in the DNA cleavage reaction and a reducer of Cu (II) followed by DMC and BDMC. The rate of formation of hydroxyl radicals by the three curcuminoids also showed a similar pattern. Curcumin is reported to be a powerful antioxidant to repair both oxidative and reductive damage caused to proteins by radiation (53).

Somparn et al (54) studied comparative antioxidant activities of curcumin, its natural demethoxy derivatives (DMC and BDMC) and metabolite hydrogenated derivatives (THC, hexahydrocurcumin; HHC, octahydrocurcumin; OHC) using DPPH and AAPH radical induced linoleic oxidation and AAPH induced red blood cell hemolysis. Results in all models demonstrated that hydrogenated derivatives of curcumin exhibited stronger scarvenging activity than curcumin and its demethoxy derivatives. Inhibitory effect of curcumin and THC on the lipid peroxidation of

erytrocyte membrane ghost at a concentration of 1.0 mg protein/ml induced by 2.0 mM tert-butylhydroperoxide. The results demonstrated that THC showed a greater inhibitory effect than curcumin especially at 150 μM (55). The *in vitro* effect of antioxidant (THC, probucal,curcumin and α-tocopherol) on 10 μM copper-induced oxidation of LDL was assessed by monitoring the formation of TBARs. All antioxidants tested dose-dependent (1-10 μM) inhibited the oxidative modification of LDL. Probucal was stronges follow by THC, α-tocopherol and curcumin. The study of the antioxidant activity of THC *in vivo*, fed rabit diets containing 1% cholesterol with or without 0.5% THC and examined their effect on oxidative stress and atherosclerosis. TBARs formation in the absence of added copper ion was inhibited in the LDL separated from THC-treated animals compared with that from control animals. THC trated to inhibit the area covered with atherosclerosis lesions compared with the control (56).

A comparative study on the antioxidant properties of THC and curcuminoids using the DPPH method, the results show that THC much more efficient than curcuminoids analogs (57). Other derivatives, DMC and BDMC also have antioxidant effect but showed weaker inhibitory activity than curcumin in the model of DPPH scarvenging radical (58), linoleic acid peroxidation (59), AAPH-induced oxidative hemolysis of human red blood cells (60) and the uptake of oxygen in the native LDL against AAPH-initiated LDL peroxidation (61).

In contrast, Ruby et al (62) have reported that Curcumin, DMC and BDMC can inhibit lipid peroxidation in a comparable level in liver homogenate induced by ascorbate and ferrous ion. Futhermore, the ability of these compounds to suppress the superoxide production by macrophages activated with phobol-12-myristate-13-acetate (PMA) indicated that all these compounds inhibited superoxide production and BDMC produced maximum effect. These results indicate that BDMC is the most active of the curcuminoids present in turmeric.

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Safety

Studies of curcumin in animals indicated that no apparent toxic effects were seen after doses of up to 5 g/kg were administered orally to Sprague–Dawley rats (37). Preclinical studies of curcumin, no toxicity has been observed from 2% dietary curcumin (approximately daily dose 1.2 g/ kg BW) administered to rats for 2 weeks (44) or from 0.2% dietary curcumin (approximately 300 mg/kg BW) administered to mice for 14 weeks (63).

Administration of 1.2–2.1 g of oral curcumin daily to patients with rheumatoid arthritis in India for 2–6 weeks did not result in any reported adverse effects (64). In a study of high dose oral curcumin in Taiwan, Cheng and colleagues administered up to 8 g daily of curcumin for 3 months to patients with pre-invasive malignant or high risk pre-malignant conditions, stating that no toxicity was observed (65). In patients with advanced colorectal cancer treated in the UK, curcumin was well tolerated at all dose levels up to 3.6 g daily for up to 4 months (66).

6. Antioxidants and their mode of action.

Antioxidants may intervene at any of the three major steps: initiation, propagation or termination of the oxidative process.

(1) Enzyme antioxidants act on specific ROS after they are formed and degrade them to less harmful products. Examples are the SODs, catalase (CAT), and GPx. SODs convert the superoxide radical to hydrogen peroxide, which is not a free radical by itself, but is a precursor of the highly reactive hydroxyl radical. Detoxification of hydrogen peroxide is carried out by

(i) CAT, which decomposes hydrogen peroxide to water and oxygen.

(ii) GPx, which reduces hydrogen peroxide to water in the presence of GSH.

The SODs, catalase, and GPx constitute the major intracellular enzymic antioxidants, while the extracellular antioxidants are mainly of the preventive and scavenging types.

(2) The preventive antioxidants act by binding to and sequestering oxidation promoters and transition metal ions, such as iron and copper, which contain unpaired electrons and strongly accelerate free radical formation. Examples of preventive antioxidants are transferrin and lactoferrin (which bind ferric ions), ceruloplasmin (which binds Cu, catalyzes the oxidation of ferrous ions to ferric due to its ferroxidase activity, and increases the binding of iron to transferrin), haptoglobins (which bind

hemoglobin), hemopexin (which binds heme), and albumin (which binds copper and heme).

(3) The scavenging or chain-breaking antioxidants act by presenting themselves for oxidation at an early stage in the free radical chain reaction and giving rise to low energy products that are unable to propagate the chain further. Lipid-soluble and water-soluble scavengers act in cellular environments that are either hydrophobic or hydrophilic, respectively. The major lipid-soluble scavengers are vitamin E (α-tocopherol), β-carotene and coenzyme Q (CoQ) while ascorbic acid, various thiols, uric acid and bilirubin function in the aqueous milieu (67).

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CHAPTER III

MATERIALS AND METHODS

1. Materials

1.1 Chemicals

All chemical were obtained commercially and used without further purification. The following chemicals were perchased from Sigma Chemical Co., St Louis, U.S.A.: Standard free cholesterol, cholesteryl arachidonate, cholesteryl linoleate, cholesteryl oleate, cholesteryl palmitate, α-tocopherol, ethylenediamine tetraacetic acid disodium salt, monobasic sodium phosphate anhydrous, disodium hydrogen phosphate anhydrous, trichloroacetic acid, 2-thiobarbituric acid, sodium dodecyl sulfate, hemin and Folin & Ciocalteu's phenol reagent.

Other chemicals were perchased from comercial sources as follow: cholesterol enzyme kit from Biotechmedical Laboratory Thailand, potassium bromide from Merck Germany, methanol, ethanol, n-hexane, butanol, acetonitrile and isopropanol (HPLC grade) from Lab Scan Co., Ltd. Thailand.

Curcumin, demethoxycurcumin, bisdemethoxycurcumin and curcuminoids (curcumin 72.29 %, demethoxycurcumin 23.50 % and bisdemethoxycurcumin 3.63%), were provided by Assistant Professor Pornchai Rojsitthisak; Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Tetrahydrocurcumin were provided by Assistant Professor Noppawan Phumala Morales; Department of Pharmacology, Faculty of Sciences, Mahidol University.

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1.2 Intruments

1) Refrigerated centrifuge (Beckman coulter model Allegra X-12R)
2) Optima Beckman Coulter ultracentrifuge (LE 80 K) with 90 Ti rotor
3) Spectrophotometer (Jasco model UVDEC 650)
4) Spectrofluorometer (Jasco model FP-777)
5) HPLC systems (shimazu, Japan) class LC 10

2. Methods

2.1 Plasma preparation

Blood was obtained from overnight fasting healthy volunteers and collected in vacutainer tubes containing EDTA 1 mg/ml blood. The plasma was separated by centrifugation at 3250 rpm for 15 min at 4 °C then stored at -80 °C until use for Low Density Lipoprotein preparation.

2.2 Low Density Lipoprotein Preparation

Low Density Lipoprotein (LDL) fractions were separated by the sequential density gradient ultracentrifugation method, which is modified from Havel method (68). LDL (density = 1.019-1.063 g/ml) was separated from plasma in salt solution by Beckman 90 Ti rotor at 50,000 rpm 4°C. Salts were removed from LDL by using dialysis bag with PBS buffer pH 7.4. LDL were then analyzed for protein content.

2.3 Protein content determination

Protein content was determined by the Markewell modification of the Lowry protein assay (69) using bovine serum albumin as standard. The aromatic amino acids residues (tyrosine and tryptophan), or the polar side chain of amino acid in the sample were reduced by the mixed acid chomagen in the reagent of Folin and Ciocalteu. The reaction could be measured by UV-visible spectrophotometer at OD 660 nm.

2.4 Preparation of hemin

Stock solution of hemin was prepared by dissolving hemin in 0.1 M NaOH and adjusted pH to 7.8 by 10 mM PBS. Hemin solution was centrifuged at 3,500 rpm for 5 min. The supernatant was collected and its concentration was determined by reading absorbance at 385 nm using molar coefficient ε = 58.4 mmol $L^{-1}cm^{-1}$. Stock solution was kept in dark at 4°C and used within a week.

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2.5 Preparation of curcuminoids, its derivetive and α-tocopherol

Curcuminoids, curcumin, DMC, BDMC, THC and α-tocopherol were dissolved in methonol. Curcuminoids, its derivetives and α-tocopherol were pipetted into the incubation tube and dried under nitrogen gas before incubation with LDL.

2.6 Other parameters measurement

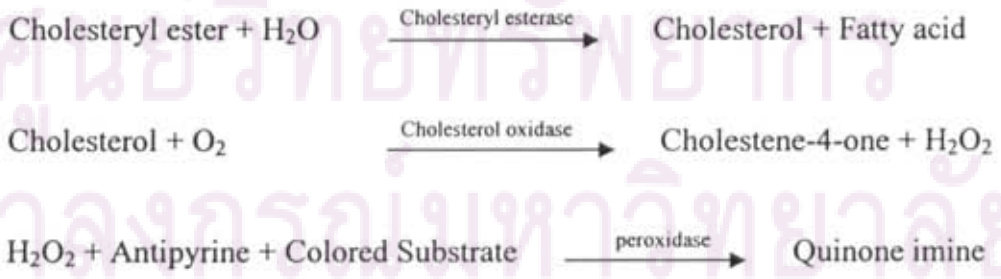
2.6.1 Thiobarbituric reactive substances (TBARs)

Lipid peroxidation of LDL 0.5 ml was terminated by adding 25 µl of 100 mM butylated hydroxytoluene. Then 0.5 ml of 10% trichloroacetic acid was added and mixed for 1 min. A 0.25 ml of 5 mM EDTA was added into the mixture. After votexing, 0.25 ml of 8% sodium dodecyl sulfate and 0.75 ml of 0.6 % thiobarbituric acid were added and votex, respectively. The reaction mixtures was then heating at 100°C for 1 hr, then samples were cooled to room temperature and measured by spectrofluorometer, excitation and emisssion wavelength at 515 and 553 nm, respectively. 1, 1, 3, 3-tetraethoxypropane was used as a standard. The results are presented as nanomoles thiobarbituric reactive substances (TBARs) per milligram protein LDL (70).

2.6.2 Total cholesterol

Total cholesterol was measured by enzymatic methods assays using commercially reagent. All sample and standard were mixed with working solution containing enzyme mixture tubes, incubated at 37 °C for 10 min and measured the absorbance at 500 nm.

Principle:



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2.6.3. Free cholesterol, Cholesteryl esters and oxidized lipid products

α-tocopherol, free cholesterol, cholesteryl esters and oxidized lipid products in LDL can be determined by reverse phase HPLC method using UV monitor at 210 nm for free cholesterol and cholesteryl esters, 234 nm for oxidized lipid products and 292 nm for α-tocopherol. This method modifies from Seta et al, Zaspel and Casllany (71,72).

Briftly, 100 µl of LDL sample was added and mixed with 200 µl of 10 mM PBS. Then 500 µl of ice-cold methanol were added and mixed on a vortex for 30 s. The 2.5 ml of hexane were added into the mixture and vortexed vigorously for 1 min and then centrifuged at 1700 rpm at 4 °C for 5 min. The 2 ml of hexane layer was transferred into a test tube and dried under nitrogen and redissolved with 200 µl of mobile phase (75% acetonitrile : 25% isopropanol, v/v). Preparation of standard substance is summarized in table 3.1. The standard mixture of α-tocopherol (5, 10 and 20 µl) and standard mixture of free cholesterol and cholesteryl esters (10, 25 and 50 µl) were injected into the hypersil BDS C18 column (5 µm: 4.6 mm x 250 mm) by autosampler. The sample was injected 50 µl. The flow rate was 1.2 ml/min and the temperature of column was controlled at 50°C. The chromatogram of free cholesterol and cholesteryl esters are shown in figure 4.1.

Table 3.1 Standard preparation of free cholesterol and cholesteryl esters

Standard substance	Stock concentration (µg/ml)	Final concentration (µg/ml)
Free cholesterol (FC)	500	60
Cholesteryl arachidonate (CA)	1000	10
Cholesteryl linoleate (CL)	500	120
Cholesteryl oleate (CO)	500	60
Cholesteryl palmitate (CP)	500	40

Free cholesterol, cholesteryl arachidonate and cholesteryl linoleate were dissolved in isopropanol. Cholesteryl oleate and Cholesteryl palmitate were dissolved in isopropanol : diethylether (5:2 v/v).

3. Experimental procedure

Part I: Study the effects of hemin induced LDL oxidation (he-oxLDL).

LDL was incubated with 2.5 μM hemin per 150 μg LDL protein at 37°C for 24 hr. He-oxLDL were collected at various time and the oxidation reaction were terminated by adding 5 mM BHT and 100 μM EDTA. Then oxidation parameters (level of TBARs and α-tocopherol) and lipid composition (total cholesterol, free cholesterol, cholesteryl esters and oxidized lipid products) were determined.

He-oxLDL were collected at various time for each parameters :

TBARs level : 0, 1, 3, 6, 9 and 24 hr.

α-Tocopherol level : 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9 and 24 hr.

Lipid composition : 0, 1, 3, 6, 9 and 24 hr.

Part II: Study the concentration dependent effects of curcuminoids and its derivatives on hemin induced LDL oxidation (he-oxLDL).

LDL were preincubated with curcuminoids, curcumin, DMC, BDMC and THC at concentrations 1, 2.5, 10, 50 and 100 μM for 30 min, α-tocopherol use as a positive control and then were incubated with 2.5 μM hemin per 150 μg LDL protein at 0 and 24 hr. Then oxidation reaction was terminated by adding 5 mM BHT and 100 μM EDTA. Then oxidation parameters (level of TBARs and α-tocopherol) and lipid composition (total cholesterol, free cholesterol, cholesteryl esters and oxidized lipid products) were determined.

Part III: Study the time dependent effect of curcuminoids and its derivatives on hemin induced LDL oxidation (he-oxLDL).

LDL were preincubated with curcuminoids, curcumin, DMC, BDMC and THC at concentrations 10 μM for 30 min, α-tocopherol use as a positive control and then were incubated with 2.5 μM hemin per 150 μg LDL protein at various incubation time. He-oxLDL were collected and the oxidation reaction were terminated by adding 5 mM BHT and 100 μM EDTA. Then oxidation parameters (level of TBARs and α-tocopherol) and lipid composition (total cholesterol, free cholesterol, cholesteryl esters and oxidized lipid products) were determined.

He-oxLDL were collected at various time for each parameters :

TBARs level: 0, 1, 3, 6, 9 and 24 hr.

α-Tocopherol level: 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9 and 24 hr.

Lipid composition: 0, 1, 3, 6, 9 and 24 hr.

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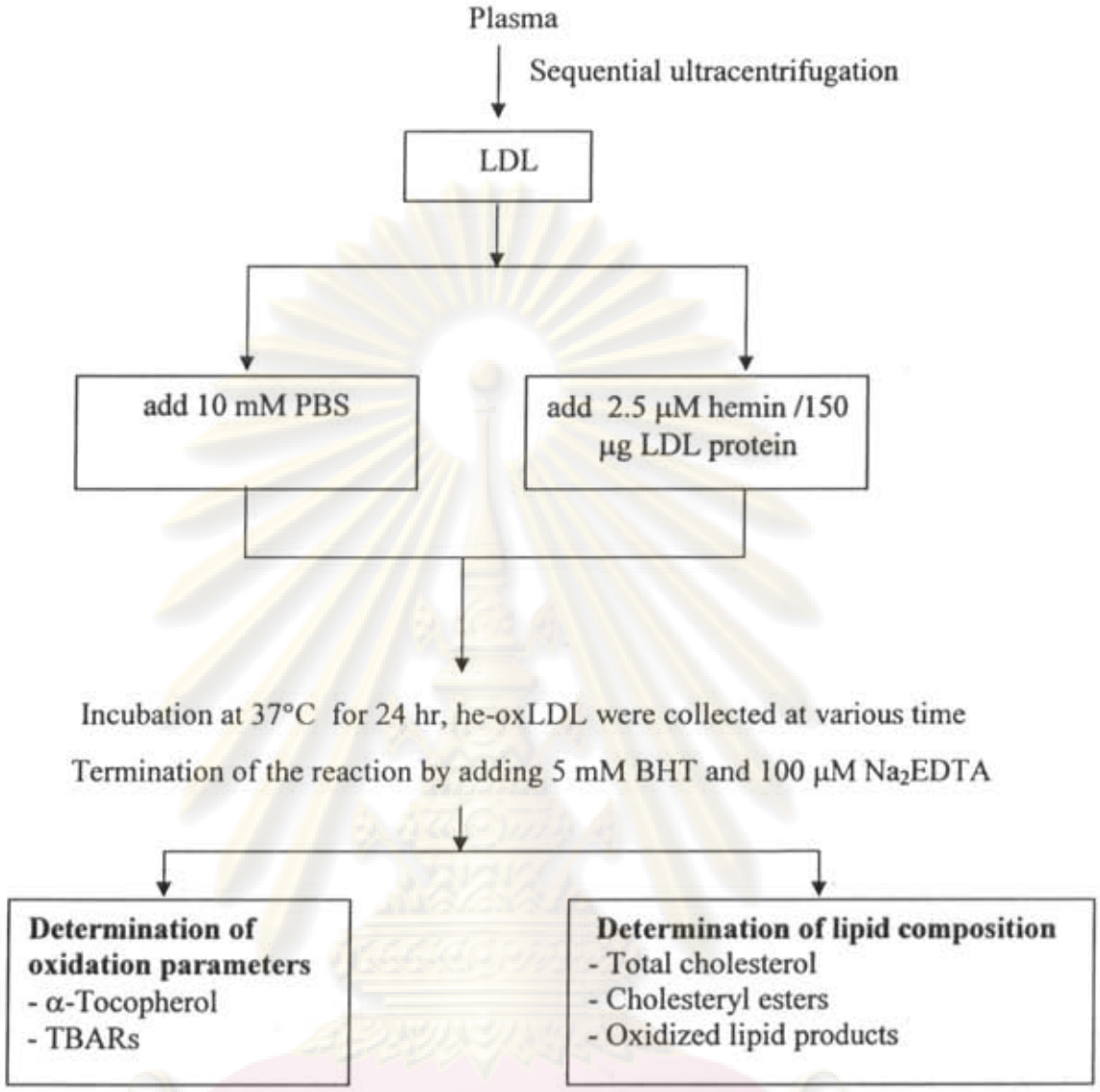

Figure 3.1 Study the effects of hemin induced LDL oxidation (he-oxLDL).

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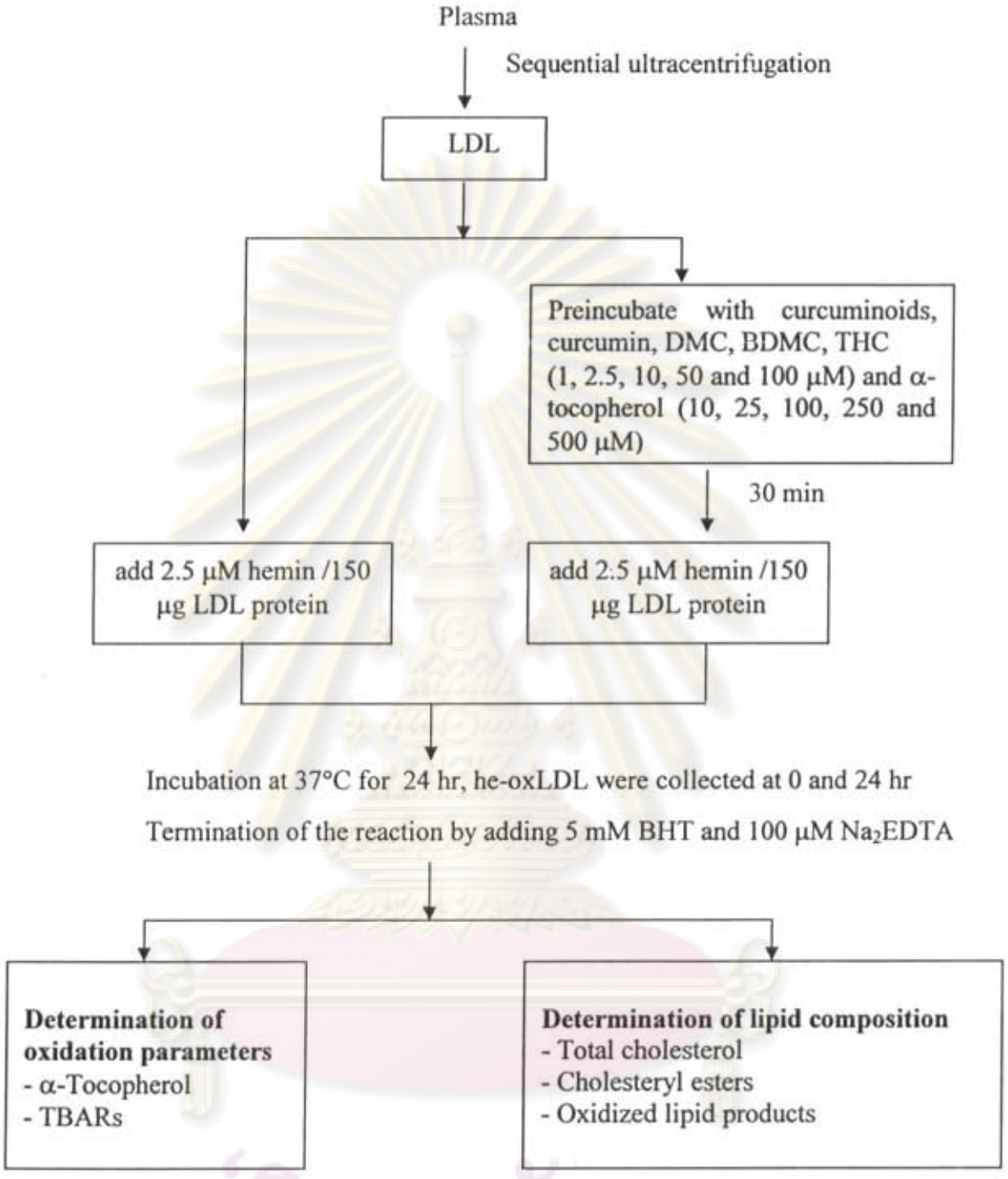

Figure 3.2 Study the concentration dependent effects of curcuminoids and its derivatives on he-oxLDL.

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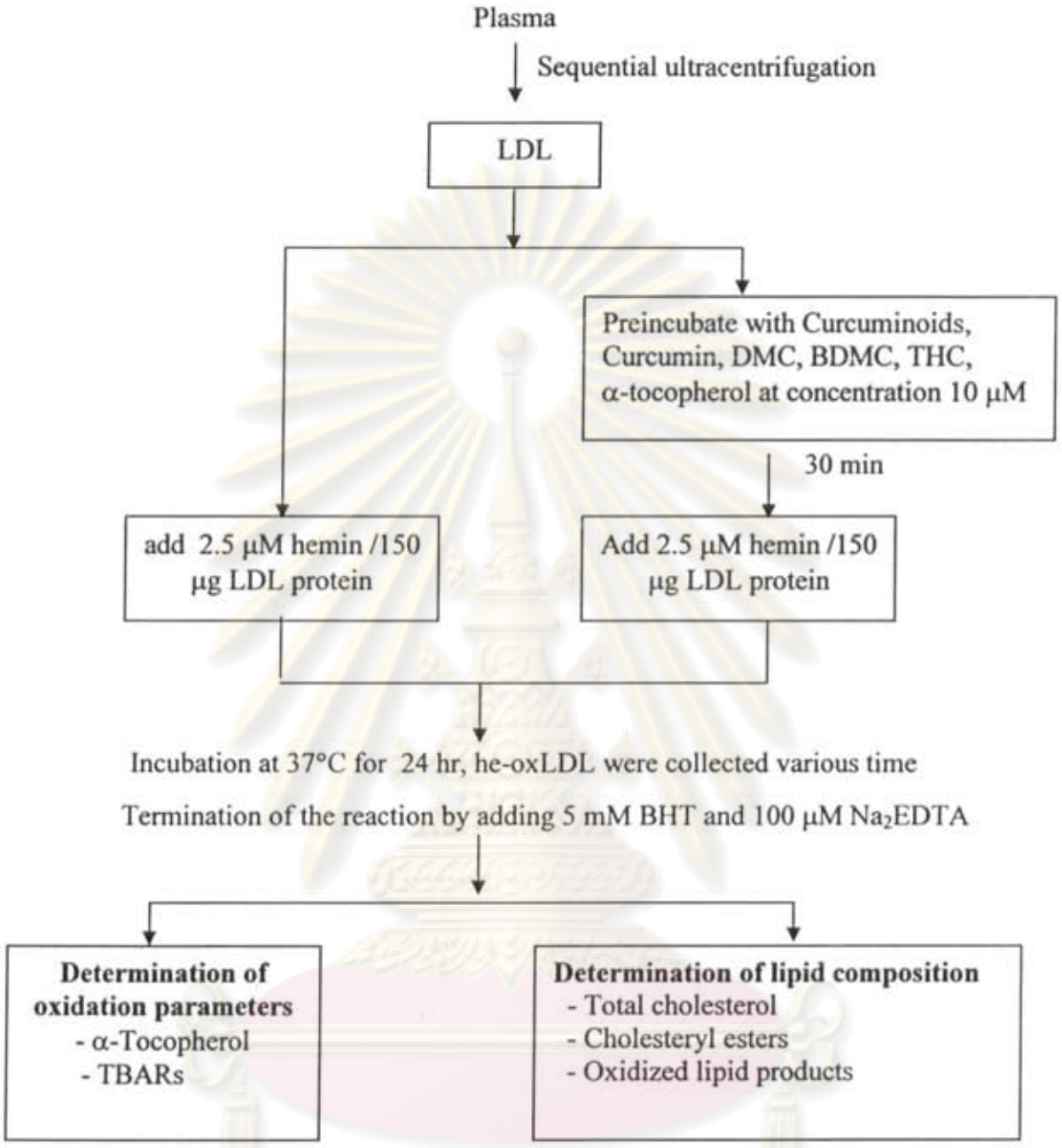

Figure 3.3 Study the time dependent effects of curcuminoids and its derivatives on he-oxLDL.

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3. Statistical Analysis

All data were presented as mean ± standard error of mean (S.E.M). Data between groups were compares by one-way analysis of varience and correlation analyses were assessed by pearson correlation using the SPSS 11 for window software. P-value less than or equal to 0.05 was acceptd as statistically significant.

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CHAPTER IV

RESULTS

Part I: The effects of hemin induced LDL oxidation (he-oxLDL).

1. α-Tocopherol and lipid composition of nLDL.

The baseline LDL levels of α-tocopherol, free cholesterol (FC), cholesteryl arachidonate (CA), cholesteryl linoleate (CL), cholesteryl oleate (CO), cholesteryl palmitate (CP) and total cholesterol were shown in table 4.1. The representative HPLC chromatogram of individual lipid was shown in figure 4.2A. The level of CL is the most abundant cholesteryl ester in LDL.

Table 4.1 Levels of α-tocopherol, free cholesterol and cholesteryl esters in nLDL.

Composition	Levels
α-Tocopherol (nmol/mg protein)	17.47 ± 0.09
Total cholesterol (μmol/mg protein)	3.62 ± 0.28
Free cholesterol (μmol/mg protein)	1.364 ± 0.064
Cholesteryl arachidonate (μmol/mg protein)	0.289 ± 0.010
Cholesteryl linoleate (μmol/mg protein)	1.340 ± 0.029
Cholesteryl oleate (μmol/mg protein)	0.511 ± 0.009
Cholesteryl palmitate (μmol/mg protein)	0.327 ± 0.015

Data were presented as mean ± S.E.M. of five independent experiments.

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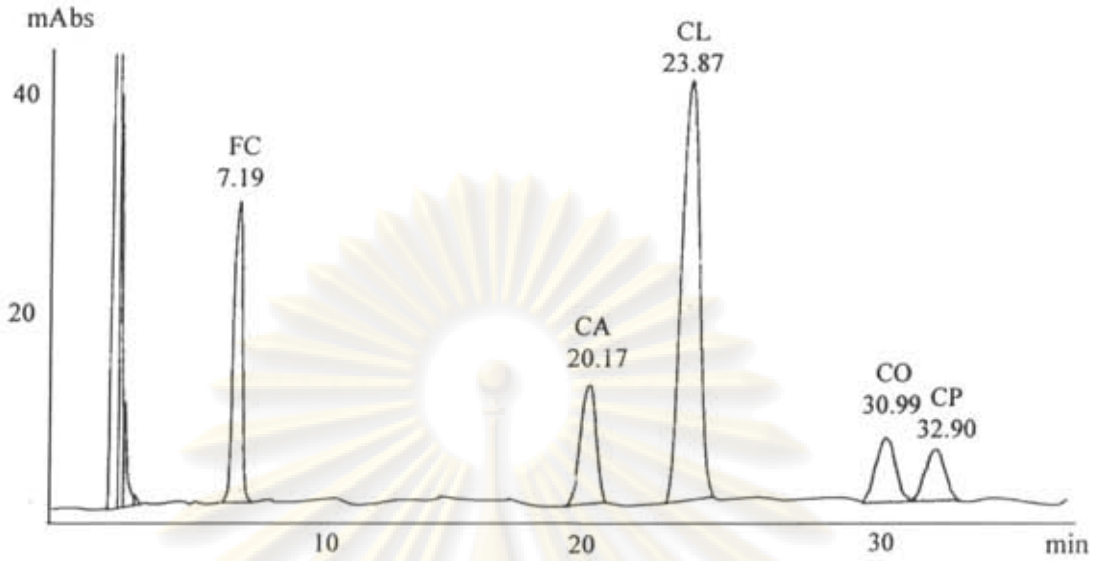

Figure 4.1 Typical HPLC chromatogram of free cholesterol (FC) and a series of cholesteryl esters standards: cholesteryl arachidonate (CA), cholesteryllinoleate (CL), cholesteryl oleate (CO) and cholesteryl palmitate (CP), determined by reverse phase HPLC method using UV monitor at 210 nm.

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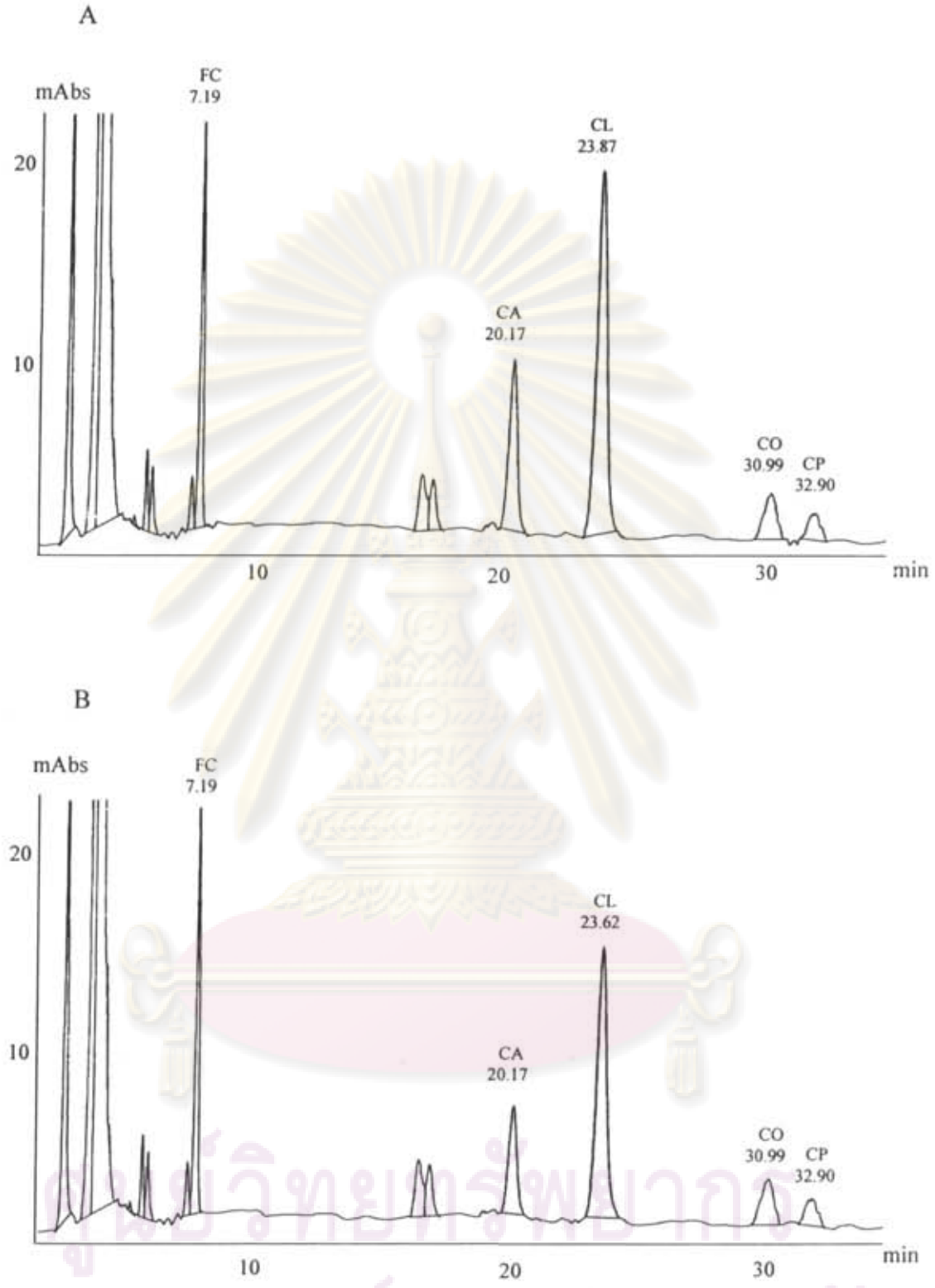

Figure 4.2 Representative HPLC chromatograms of free cholesterol and cholesteryl esters in nLDL (A) and he-oxLDL (B) that induced by 2.5 μM hemin at 24 hr of incubation, determined by reverse phase HPLC method using UV monitor at 210 nm.

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2. The effect of hemin on TBARs formation and α-tocopherol levels.

TBARs is a marker to determine lipid peroxidation products. We found that the TBARs levels in nLDL were not significantly changed when incubation at 37 °C for 24 hr (2.0 ± 0.07 *v.s* 2.79 ± 0.16 nmol/mg protein). However, when incubation LDL with 2.5 μM hemin, TBARs levels were slightly increased during 0-3 hr and rapidly increased from 3 hr (3.7 ± 0.10 nmol/mg protein) until almost reach the maximum levels at 9 hr of incubation (19.6 ± 1.02 nmol/mg protein) (Figure 4.3). The TBARs levels at 9 and 24 hr were not significant different. The results indicated that hemin can induce lipid peroxidation in LDL.

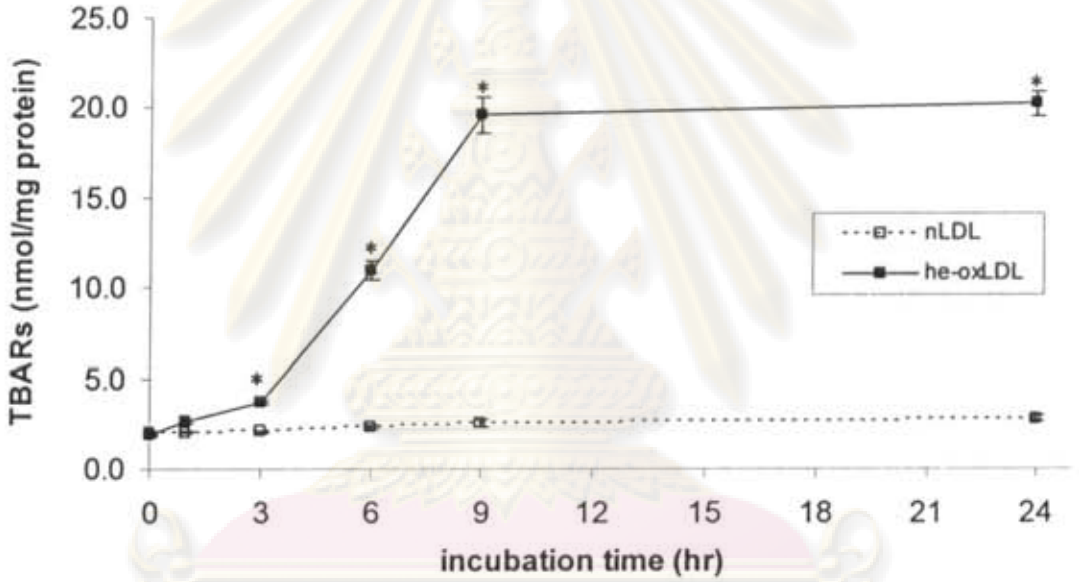

Figure 4.3 Effect of 2.5 μM hemin on the TBARs formation in LDL oxidation. *Significant different at $p<0.05$ compared to respective nLDL control (n =5).

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α-Tocopherol is the major lipid soluble endogenous antioxidant in LDL. The time course effect of he-oxLDL on levels of α-tocopherol was shown in figure 4.4. The levels of α-tocopherol in he-oxLDL was rapidly decreased during 0-3 hr and decreased until undetectable at 5 hr of incubation. The depletion of α-tocopherol indicated that α-tocopherol was consumed during hemin induced LDL oxidation.

The inverse correlation between level of TBARs and α-tocopherol in he-oxLDL was found with the r-value of –0.935 ($p<0.01$) from 0-3 hr (figure 4.5A). While α-tocopherol was depleted until undetectable, the TBARs level were dramatically increased, the correlation disappear (figure 4.5B) indicating that α-tocopherol protect the LDL from the lipid peroxidation induced by hemin especially at the 0-3 hr of incubation (this period can be called as lag phase).

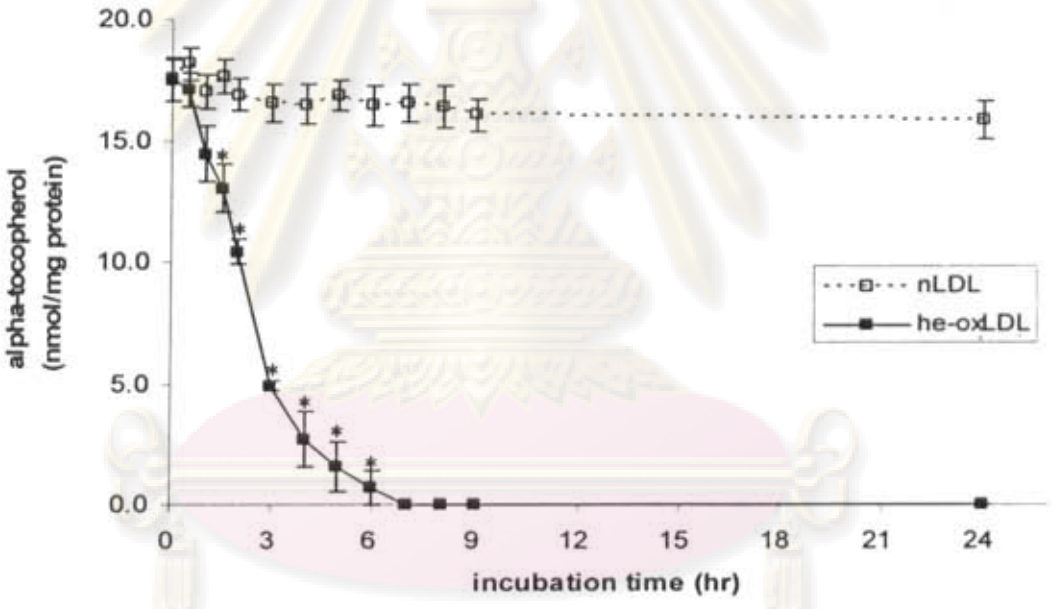

Figure 4.4 Effect of 2.5 μM hemin on α-tocopherol levels in LDL oxidation. *Significant different at $p<0.001$ compared to respective nLDL control ($n = 5$).

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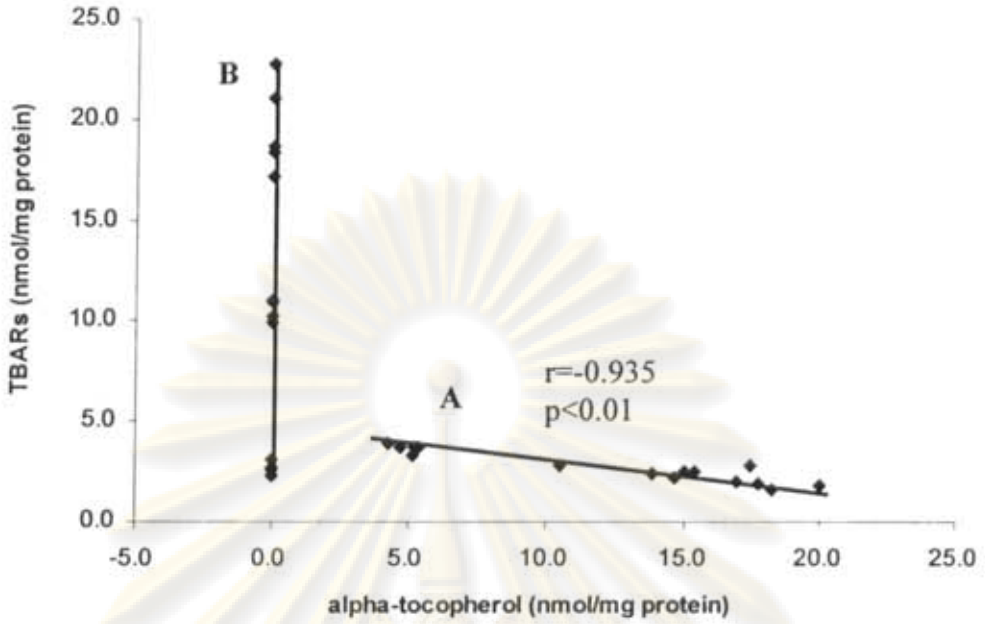

Figure 4.5 The correlation between the level of TBARs and α-tocopherol during 0-3 hr (A) and 6-24 hr (B) of incubation LDL with 2.5 μM hemin. The data analyses from five independent experiments.

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3. The effect of hemin on levels of free cholesterol and cholesteryl esters.

The effect of hemin induced LDL oxidation on levels of free cholesterol and cholesteryl esters were shown in figure 4.6 and figure 4.7. Levels of CA and CL were decreased while levels of FC, CO and CP were not changed after incubation with hemin. The representative HPLC chromatogram of individual lipid was shown in figure 4.2B. At 24 hr incubation of hemin, the levels of CA and CL were significantly decreased 28.9% and 24.2% ($p<0.05$) from nLDL (figure 4.6 A and B respectively).

The percent decrease of CA and CL show a good correlation with levels of TBARs ($r=0.857$, $p<0.01$ and 0.854, $p<0.01$ respectively) (Figure 4.8A-B). The correlation indicated that CA and CL were the major substrates of lipid peroxidation for the formation of TBARs.

The decrease ratio of cholesteryl linoleate to cholesteryl oleate (CL/CO) is known to reflect increased free radical production (80). Our result revealed that the CL/CO ratio in nLDL were not changed when incubation at 37 °C for 24 hr (2.6 ± 0.07 *v.s* 2.5 ± 0.06). The CL/CO ratio in he-oxLDL was significantly decreased at 6, 9 and 24 hr of incubation when compared to the nLDL (2.2 ± 0.09 *v.s.* 2.6 ± 0.10, $p<0.05$, 2.0 ± 0.06 *v.s.* 2.6 ± 0.10 and 1.9 ± 0.09 *v.s.* 2.5 ± 0.06, $p<0.001$ respectively) (figure 4.9). We also found the inverse correlation between the level of TBARs and CL/CO ratio in he-oxLDL with the r-value of –0.682 ($p<0.01$) (figure 4.10). So our result indicated that hemin can cause lipid peroxidation in the time course-effect manner and the more decreased of CL/CO ratio, the more damaged of lipid. According to the previous study that a CL/CO ratio of less than 2 could be used as a critical point to determine that lipoproteins were damaged (12).

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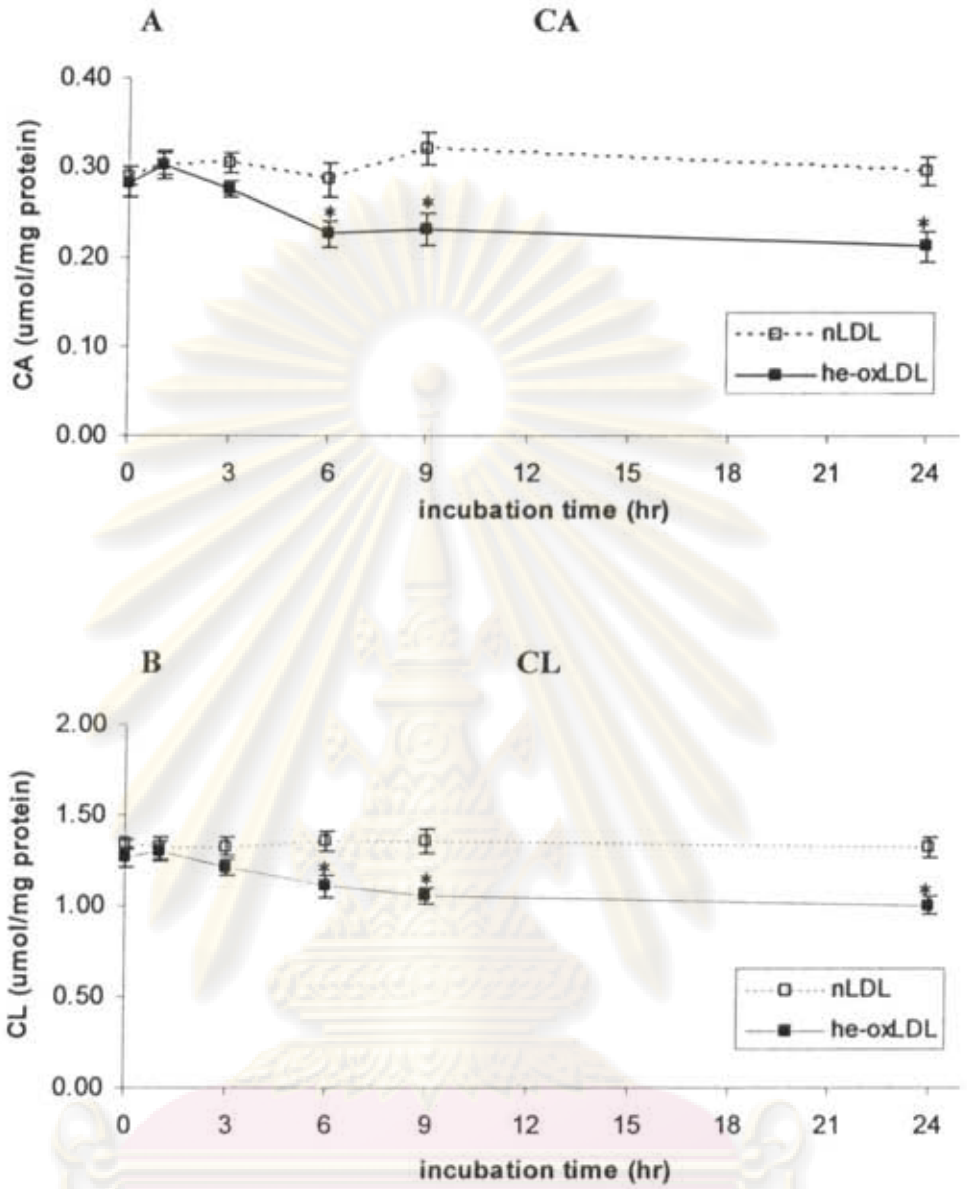

Figure 4.6 Effect of 2.5 μM hemin on levels of (A) cholesteryl arachidonate (CA) and (B) cholesteryl linoleate (CL) in LDL oxidation. *Significant different at $p<0.05$ compared to respective nLDL control (n = 5).

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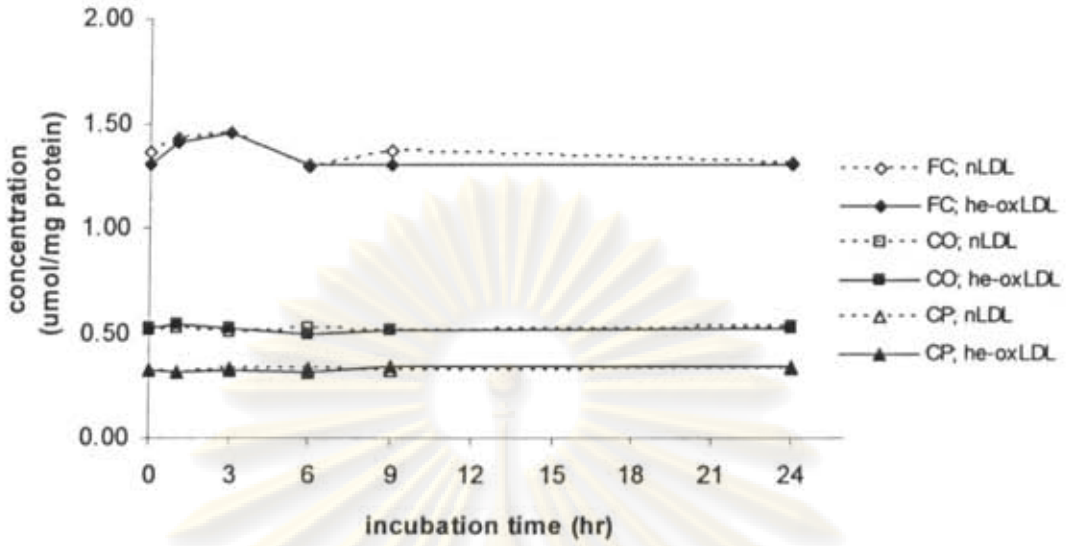

Figure 4.7 Effect of 2.5 μM hemin on levels of free cholesterol (FC), cholesteryl oleate (CO) and cholesteryl palmitate (CP) in nLDL and he-oxLDL (n = 5).

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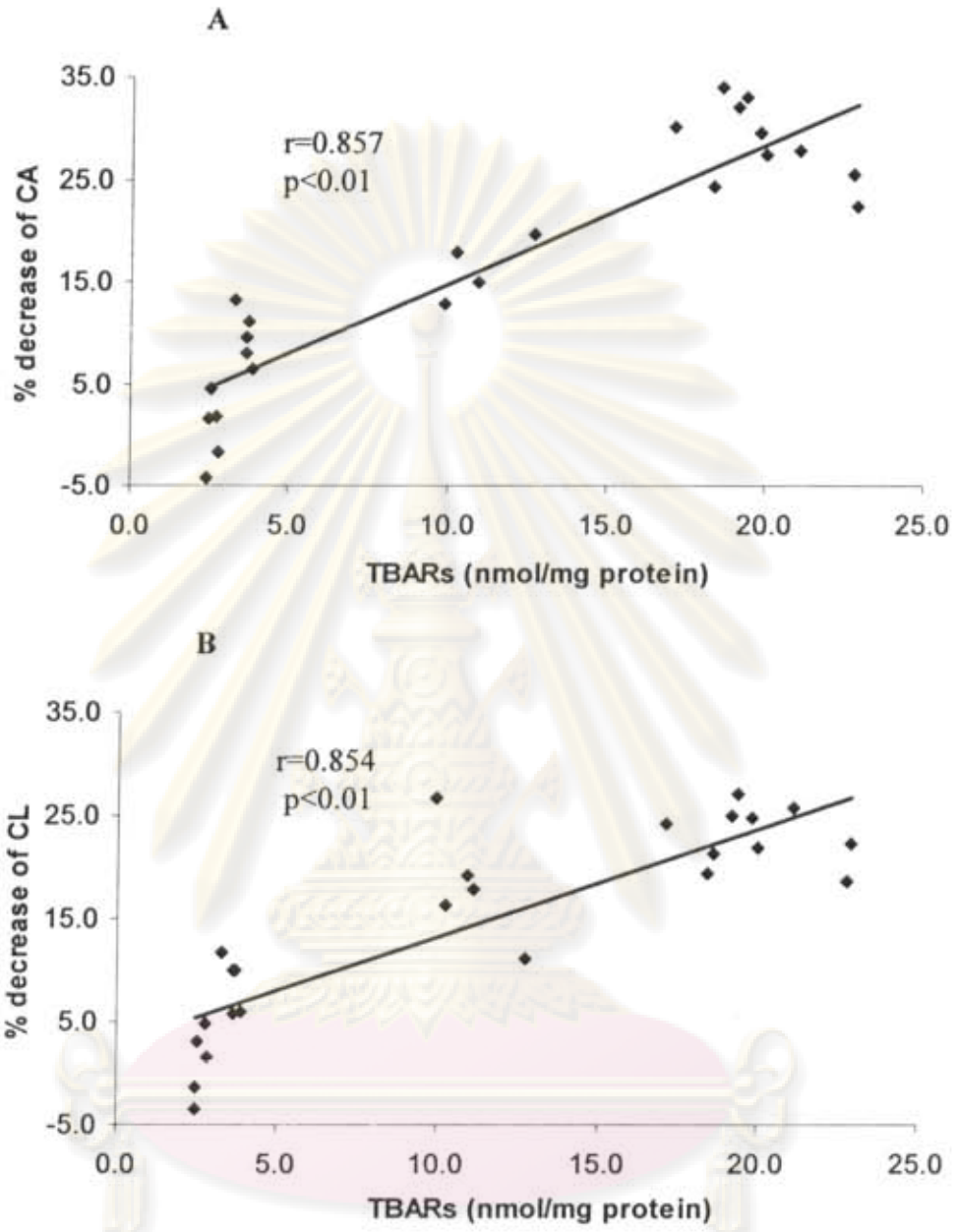

Figure 4.8 The correlation between TBARs with percent decrease of CA (A) and CL (B). The data analyses from five independent experiments.

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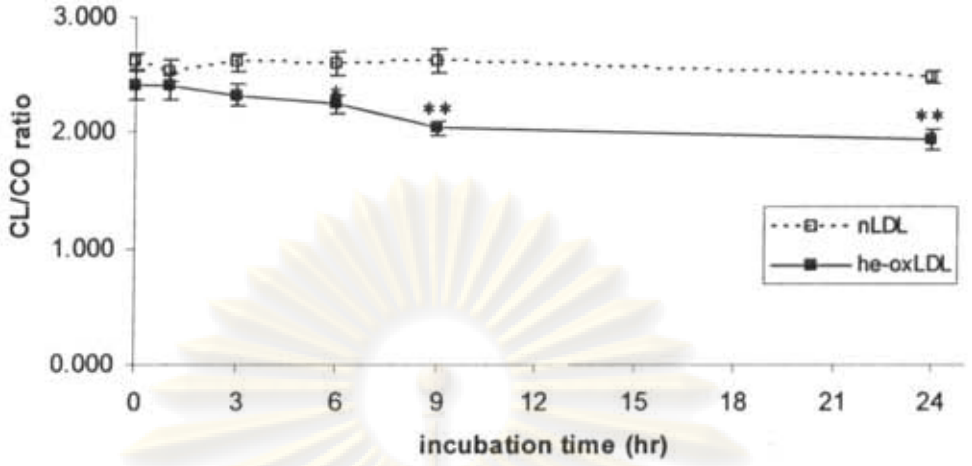

Figure 4.9 Effect of 2.5 μM hemin on CL/CO ratio in LDL oxidation. *Significant different at *p<0.05, **Significant different at p<0.001 compared to respective nLDL control (n = 5).

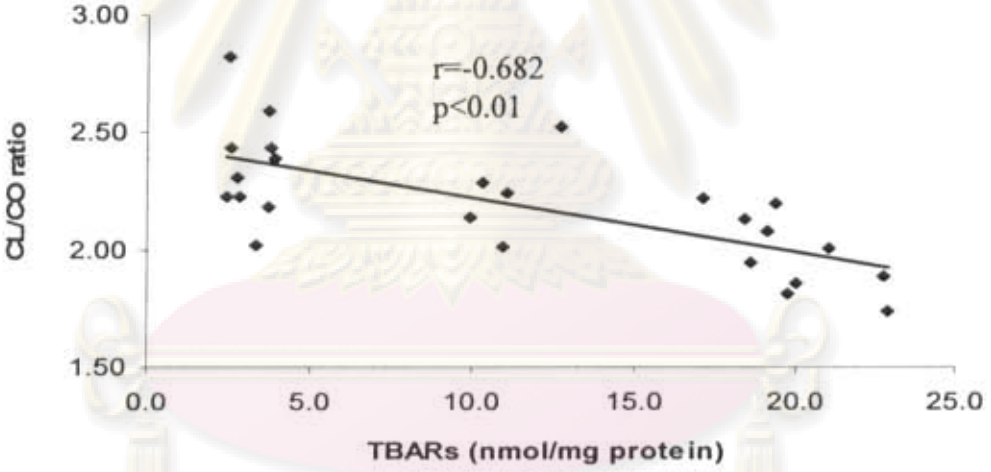

Figure 4.10 The correlation between TBARs and CL/CO ratio. The data analyses from five independent experiments.

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4. The effect of hemin on oxidized lipid products formation.

The oxidized lipid products formation were determined by HPLC-UV-detector 234 nm. Peaks at retention time (RT) of 9.6, 10.3, 11.6 and 12.3 min were detected during LDL oxidation (figure 4.11). These peaks were unknown oxidized lipid products due to there unavailable standard. Figure 4.12 shows the time course effect of 4 oxidized lipid products which plot between area under the curve (AUC) and incubation time of LDL oxidation. We found that oxidized lipid products formation at RT 9.6 min was the highest level follow by RT 12.3 min while RT 10.3 min has a comparable level with RT 11.6 min. In addition, the results showed that the oxidized lipid products at RT 9.6 and 12.3 min were slightly increased during 0-3 hr and rapidly increased from 3 hr until almost reach the maximum levels at 9 hr of incubation. The oxidized lipid products levels at 9 and 24 hr were not significant different ($p<0.05$).

Area under the curve of each oxidized lipid product peaks showed a good correlation with TBARs levels, % decrease CA and CL (Table 4.2). These correlation suggested that the oxidized lipid products formation come from the lipid peroxidation of CA and CL.

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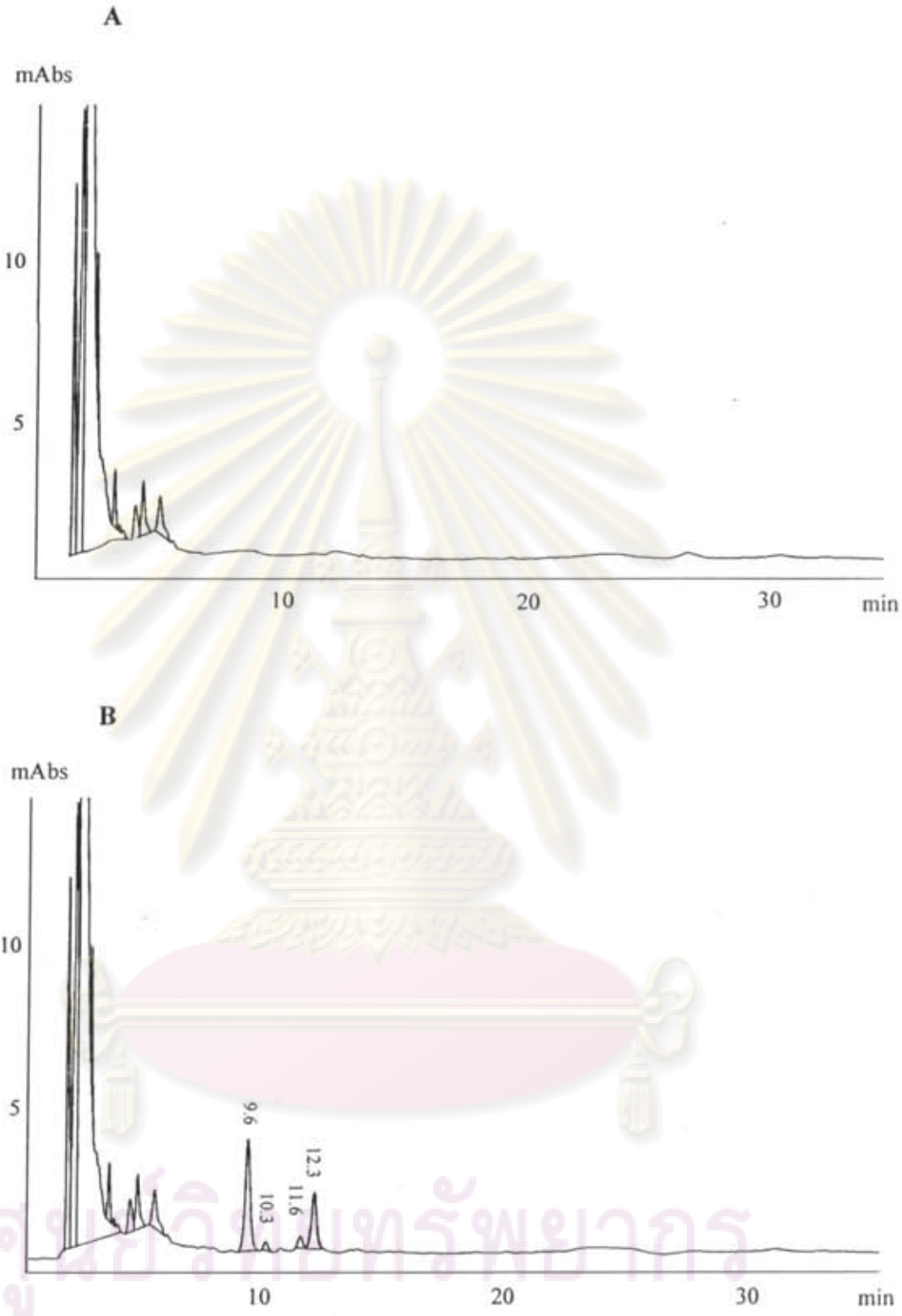

Figure 4.11 Representative HPLC chromatograms of oxidized lipid products in nLDL (A) and he-oxLDL (B) that induced by 2.5 μM hemin at 24 hr of incubation, determined by reverse phase HPLC method using UV monitor at 234 nm.

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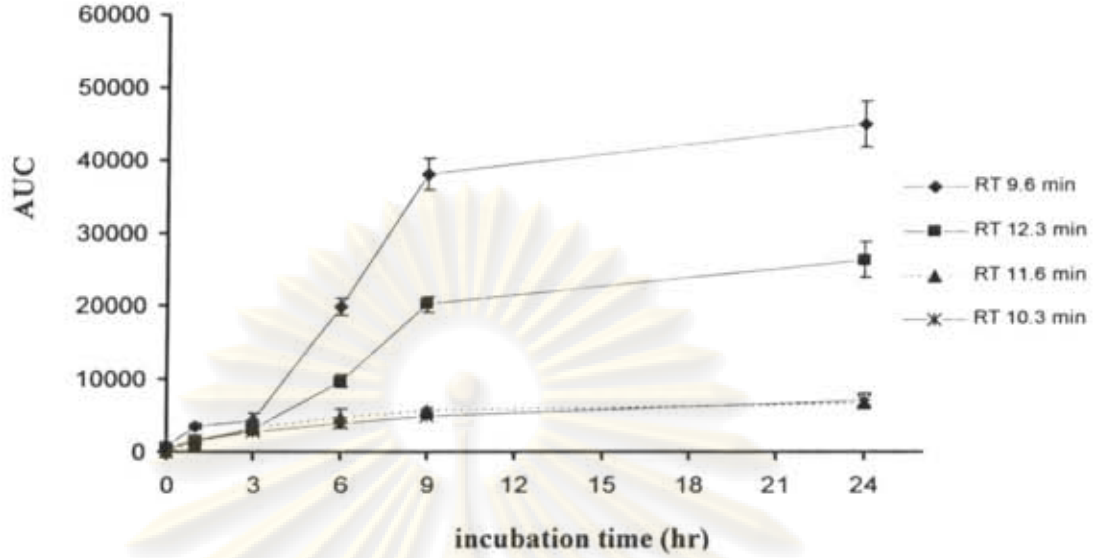

Figure 4.12 Oxidized lipid products formation in he-oxLDL (n = 5).

Table 4.2 The significant correlation of oxidized lipid products with oxidation and lipid composition parameters in the he-oxLDL (significant correlation at $p < 0.01$).

Oxidation and lipid composition parameters	r-values			
	Oxidized lipid products			
	RT 9.6 min	RT 10.3 min	RT 11.6 min	RT12.3 min
TBARs	0.963	0.804	0.800	0.946
% decrease of CA	0.805	0.727	0.742	0.755
% decrease of CL	0.871	0.700	0.709	0.791

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Part II: The study of the concentration dependent effects of curcuminoids and its derivatives on hemin induced LDL oxidation (he-oxLDL).

1. The effect of curcuminoids and its derivatives on TBARs formation and α-tocopherol levels.

The curcuminoids, curcumin, DMC, BDMC, THC and α-tocopherol were able to inhibit TBARs formation. All tested compounds possess the inhibitory effect on lipid peroxidation in concentration dependent manner (figure 4.13). The 50% inhibition concentration (IC_{50}) was obtained from the plot between % inhibition of TBARs formation and concentration of tested compounds was shown in table 4.3. The result indicated that curcumnoids and its derivatives had much more protective effect than α-tocopherol, a positive control. A significant protective effect on TBARs formation is in the order of THC ≥ curcumin ≥ curcuminoids ≥ DMC > BDMC > α-tocopherol ($p<0.05$).

Normally α-tocopherol in hemin induced LDL oxidation was decreased until disappear but when adding each tested compound, α-tocopherol remain in LDL at 24 hr of incubation approximately 22-50% depend on the concentration of tested compounds. The concentration of tested compound to protect the decreasing of α-tocopherol for curcumin and THC were 10, 50 and 100 μM, curcuminods were 50 and 100 μM and DMC was 100 μM but BDMC can not protect at all test concentrations (Table 4.4).

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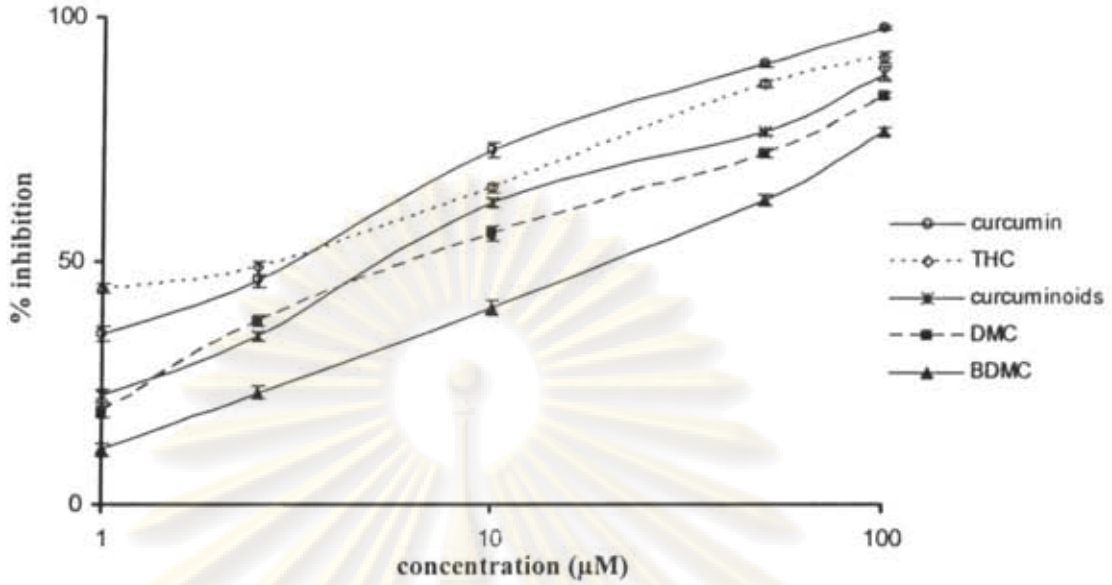

Figure 4.13 The percent inhibition of curcumin, DMC, BDMC, curcuminoids and THC on TBARs formation at 24 hr of incubation LDL with hemin. The lines were acquired by plotting the concentrations of tested compounds (1, 2.5, 10, 50 and 100 μM) against the percent inhibition of TBARs formation (n = 5).

Table 4.3 IC_{50} values of curcuminoids, its derivatives and α-tocopherol on TBARs formation at 24 hr of incubation LDL with hemin.

Tested compounds	IC_{50} (μM)
curcumin	$^{a}2.7 \pm 0.2$
DMC	$^{b}7.9 \pm 0.5$
BDMC	$^{c}18.8 \pm 1.0$
curcuminoids	$^{ab}6.3 \pm 0.3$
THC	$^{a}2.2 \pm 0.2$
α-tocopherol	$^{d}69.4 \pm 3.9$

Data were presented as mean ± S.E.M. of five independent experiments. Means with the same letter are not significantly different.

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Table 4.4 The effect of curcuminoids and its derivatives on % remainder of α-tocopherol at 24 hr of incubation LDL with hemin.

Tested compounds	% remainder of α-tocopherol				
	1 μM	2.5 μM	10 μM	50 μM	100 μM
curcumin	0	0	22.4 ± 1.3	30.2 ± 0.4	40.7 ± 0.9
DMC	0	0	0	0	30.2 ± 1.5
BDMC	0	0	0	0	0
curcuminoids	0	0	0	24.2 ± 6.1	28.6 ± 7.1
THC	0	0	24.7 ± 0.8	30.8 ± 1.5	49.4 ± 2.1

Data were presented as mean ± S.E.M. of five independent experiments.

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2. The effect of curcuminoids and its derivatives on cholesteryl esters.

The curcuminoids, curcumin, DMC, BDMC, THC and α-tocopherol were able to protect the decrease of CA and CL in he-oxLDL. All tested compounds had protective effect in concentration dependent manner (figure 4.14 A and B). The IC_{50} was obtained from the plot between % inhibition of decreasing CA and CL with concentrations of tested compounds (table 4.5). The result indicated that curcuminoids and its derivatives had more effective than α-tocopherol to protect the damaged of lipid composition. A significant protective effect on decrease of CA and CL is in the order of THC ≥ curcumin ≥ curcuminoids > DMC > BDMC > α-tocopherol ($p<0.05$).

In addition, all tested compounds were able to protect the decrease of CL/CO ratio in he-oxLDL in concentration dependent manner (figure 4.15). The IC_{50} was obtained from the plot between % inhibition of decreasing CL/CO ratio and concentrations of tested compounds (table 4.5). A significant protective effect on decrease of CL/CO ratio is in the order of THC ≥ curcumin ≥ curcuminoids > DMC > BDMC > α-tocopherol ($p<0.05$), similarly the protective effect on TBARs formation and decrease of CA and CL.

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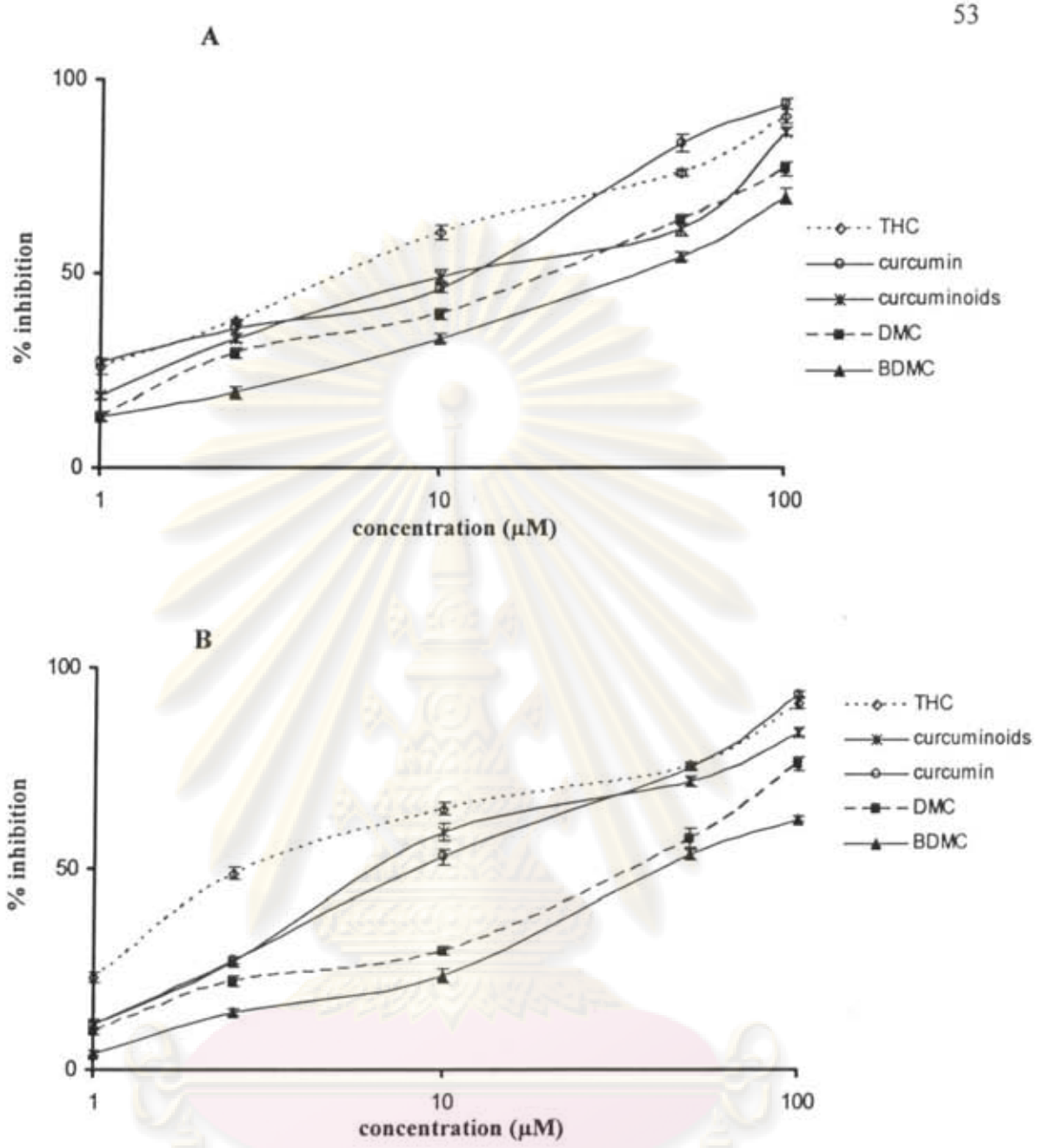

Figure 4.14 The percent inhibition of curcumin, DMC, BDMC, curcuminoids and THC on decreasing CA and CL at 24 hr of incubation LDL with hemin. The lines were acquired by plotting the concentrations of tested compounds (1, 2.5, 10, 50 and 100 μM) against the percent inhibition of decreasing CA (A) and CL (B) (n = 5).

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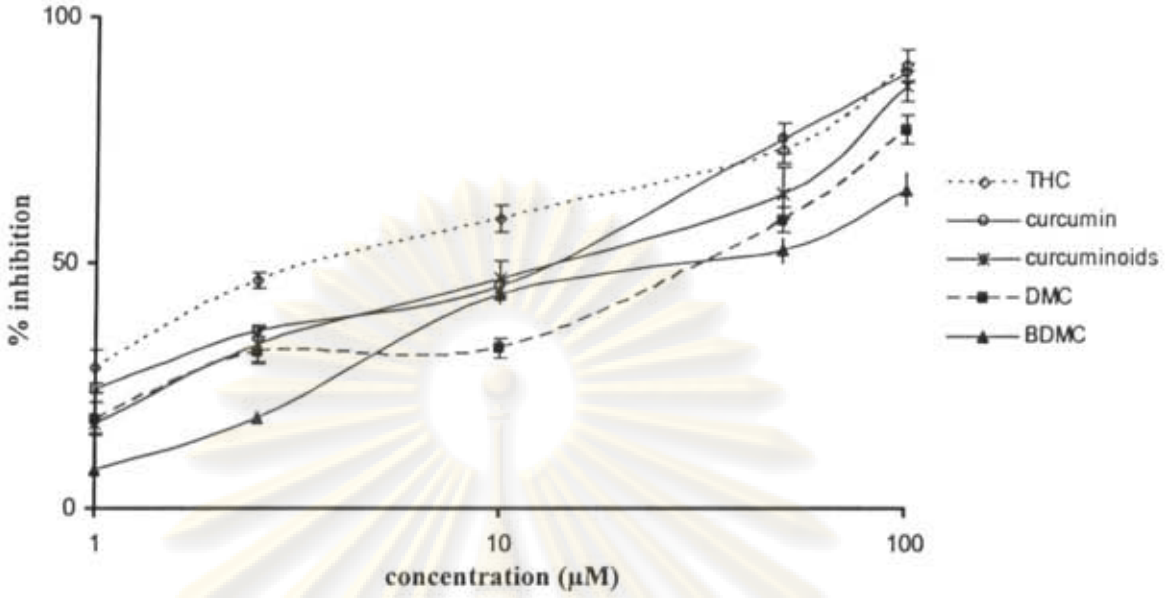

Figure 4.15 The percent inhibition of curcumin, DMC, BDMC, curcuminoids and THC on decreasing CL/CO ratio at 24 hr of incubation LDL with hemin. The lines were acquired by plotting the concentrations of tested compounds (1, 2.5, 10, 50 and 100 μM) against the percent inhibition of decreasing CL/CO ratio (n = 5).

Table 4.5 IC_{50} values of curcuminoids, its derivatives and α-tocopherol on decreasing CA, CL and CL/CO ratio at 24 hr of incubation LDL with hemin.

Tested compounds	IC_{50} (μM) decreasing		
	CA	CL	CL/CO ratio
curcumin	^{a}9.3 ± 0.4	^{ab}6.0 ± 0.6	^{a}7.7 ± 0.7
DMC	^{b}25.5 ± 1.2	^{c}16.5 ± 0.7	^{c}19.1 ± 1.9
BDMC	^{c}47.3 ± 1.5	^{d}30.0 ± 1.6	^{d}33.8 ± 5.5
curcuminoids	^{a}10.4 ± 0.9	^{b}10.8 ± 0.9	^{ab}11.7 ± 1.8
THC	^{a}4.5 ± 0.3	^{a}5.5 ± 0.4	^{a}5.0 ± 0.9
α-tocopherol	^{d}65.9 ± 5.2	^{c}60.4 ± 25	^{c}58.6 ± 12.0

Data were presented as mean ± S.E.M. of five independent experiments.
Means with the same letter are not significantly different.

3. The effect of curcuminoid, its derivatives and α-tocopherol on oxidized lipid products.

The study of the effects of he-oxLDL in part I showed that oxidized lipid products were occurred at retention time (RT) 9.6, 10.3, 11.6 and 12.3 min. The study in this part found that curcumin, DMC, BDMC, curcuminoids, THC and α-tocopherol were able to inihibit oxidized lipid products formation in concentration dependent manner at RT 9.6 and 12.3 min (figure 4.16 A and D). While at RT 10.3 and 11.6 min, all tested compounds at 2.5, 10, 50 and 100 μM completely inhibited oxidized lipid products formation (Figure 4.16 B and C).

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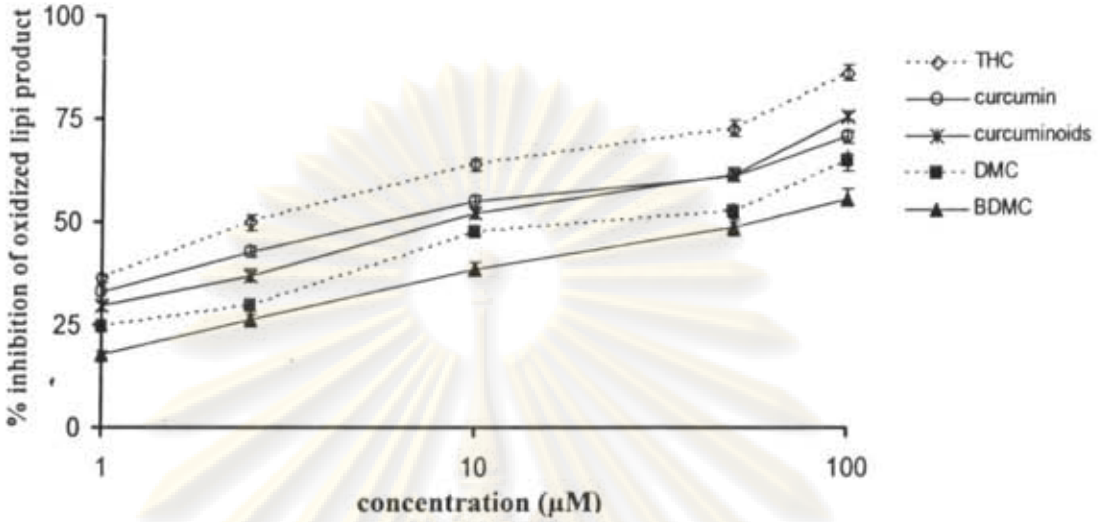
A Oxidized lipid product at RT 9.6 min



B Oxidized lipid product at RT 10.3 min

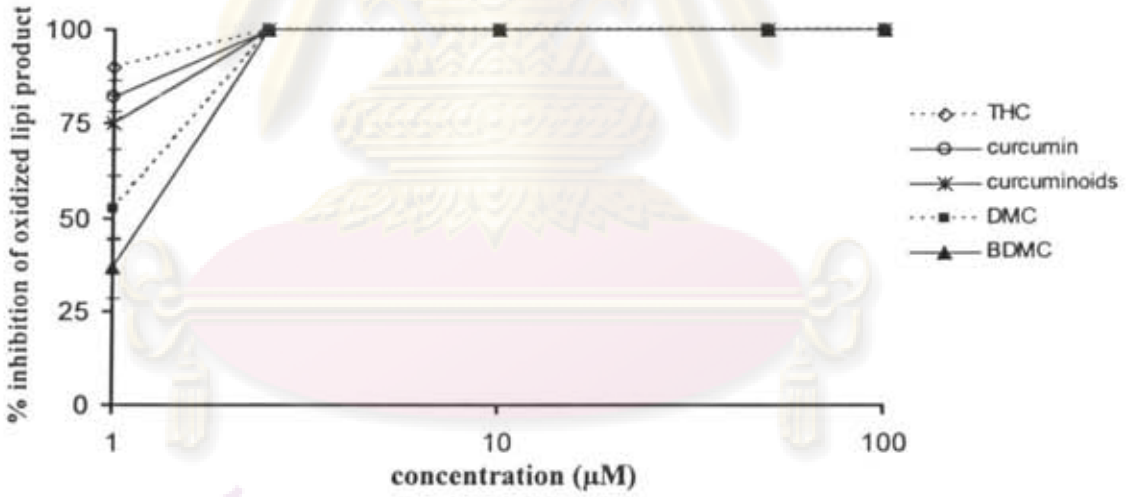


Figure 4.16 cont

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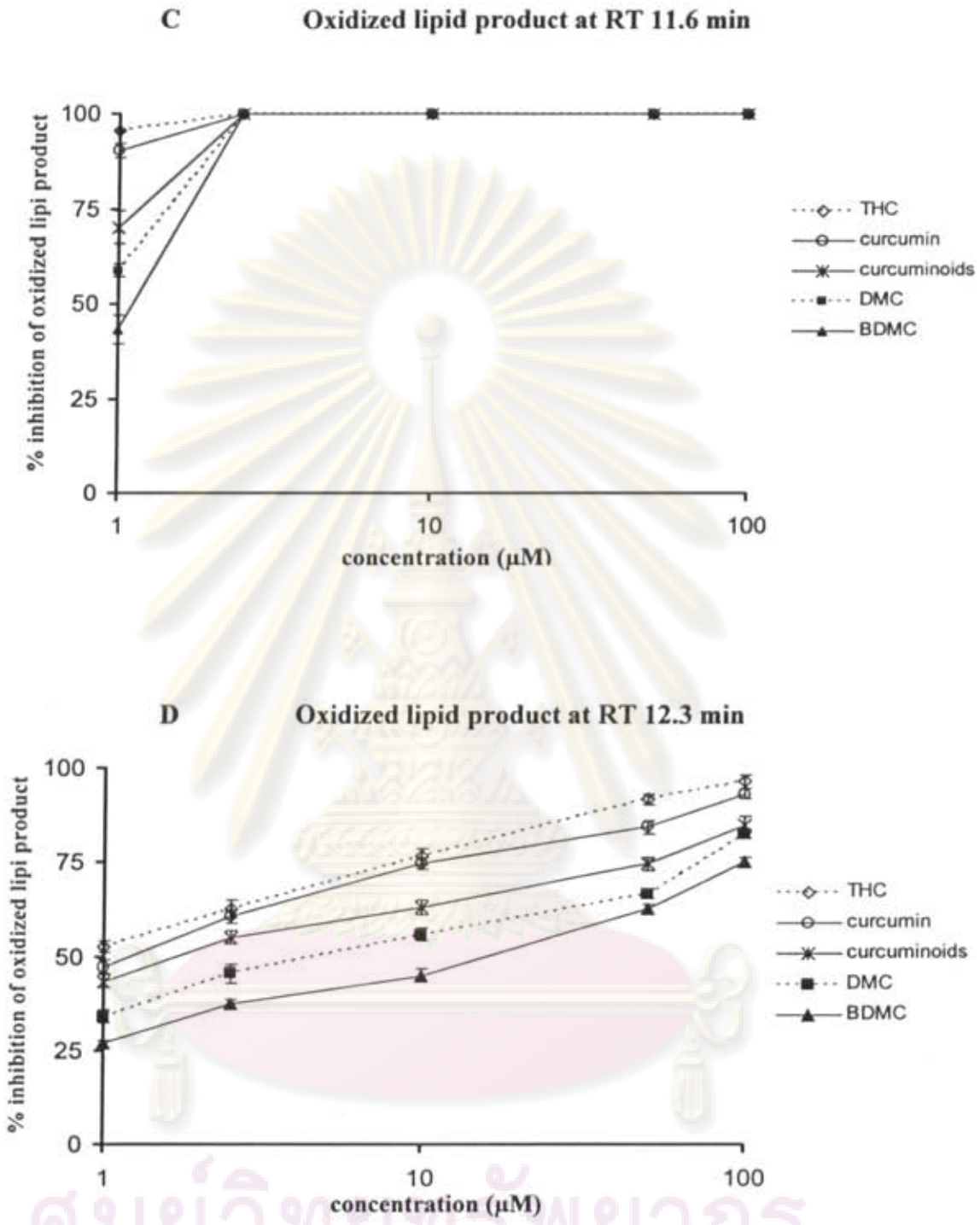

Figure 4.16 The percent inhibition of curcumin, DMC, BDMC, curcuminoids and THC on oxidized lipid products formation at 24 hr of incubation LDL with hemin (n = 5).

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Part III: The study of the time dependent effect of curcuminoids and its derivatives on hemin induced LDL oxidation (he-oxLDL).

1. The effect of curcuminoids and its derivatives on TBARs formation and α-tocopherol levels.

The l0 μM curcuminoids, curcumin, DMC, BDMC, THC and α-tocopherol were able to inhibit the rate of TBARs formation in he-oxLDL along 24 hr (figure 4.17).

The rate of TBARs formation in he-oxLDL have 3 phase, first phase was in 0-3 hr with the rate of 0.579 nmol/mg protein hr^{-1}, second phase was in 3-9 hr with the rate of 2.655 nmol/mg protein hr^{-1} and third phase was in 9-24 hr with the rate of 0.119 nmol/mg protein hr^{-1}. Rate of TBARs formation and inhibition rate (R_{inh}) of TBARs formation were shown in table 4.6 and 4.7, respectively. At first and second phase, THC was the most potent to inhibit TBARs formation compared to another tested compounds. The third phase that reach the maximum lipid peroxidation, the rate of TBARs formation of tested compounds were very low and were not different from he-oxLDL (table 4.6).

The level of α-tocopherol in he-oxLDL was decreased until undetectable at 5 hr of incubation. Curcuminoids and its derivatives were able to prolong the time to maintain α-tocopherol levels. BDMC and DMC can maintain only 8 and 9 hr, respectively while curcuminoids, curcumin and THC were able to maintain the α-tocopherol levels throughout 24 hr (figure 4.18).

The decreasing rate of α-tocopherol and R_{inh} of decreasing α-tocopherol were shown in table 4.8 and 4.9, respectively. In first phase, decreasing rate of α-tocopherol in he-oxLDL was 6.54×10^{-2} nmol/mg protein min^{-1}, second phase was 3.38×10^{-2} nmol/mg protein min^{-1} and when lipid peroxidation reach the maximum levels, the decreasing rate of α-tocopherol disappear. At first and second phase, THC was more potent to inhibit the rate decreasing α-tocopherol than another tested compounds. The third phase that α-tocopherol in he-oxLDL was disappeared, the rate decreasing of α-tocopherol were very low.

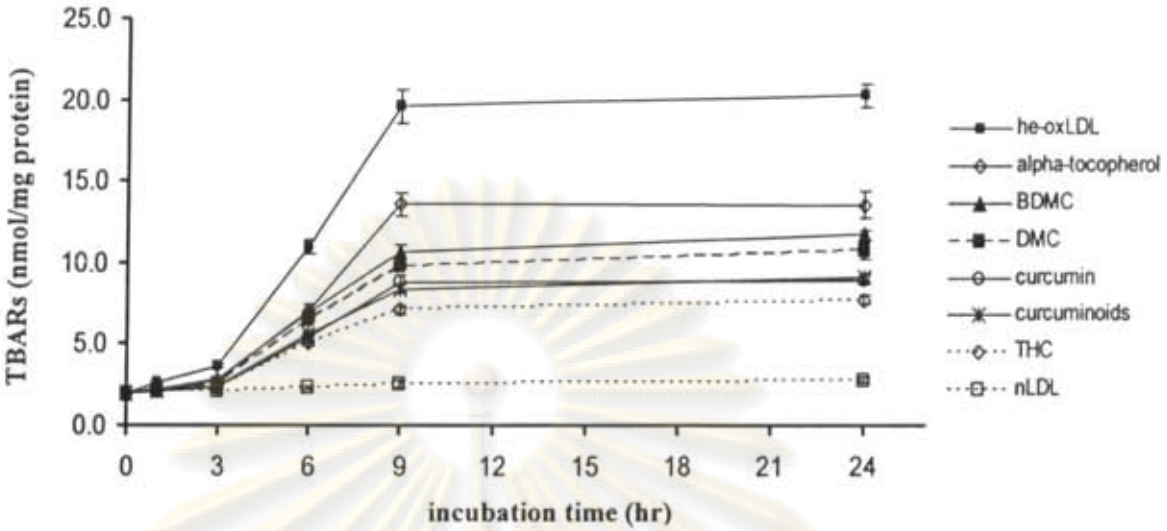

Figure 4.17 The time course effect of 10 μM curcumin, DMC, BDMC, curcuminoids, THC and α-tocopherol on levels of TBARs formation in ox-LDL during incubated with hemin (n = 5).

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Table 4.6 Effect of 10 μM curcuminoids, its derivatives and α-tocopherol on rate of TBARs formation in he-oxLDL.

condition	rate of TBARs formation (nmol/mg protein hr^{-1})		
	0-3 hr	3-9 hr	9-24 hr
nLDL	^{a}0.029 ± 0.025	^{a}0.074 ± 0.023	^{a}0.017 ± 0.004
he-oxLDL	^{b}0.579 ± 0.043	^{b}2.655 ± 0.169	^{a}0.119 ± 0.098
curcumin	^{ad}0.108 ± 0.035	^{dc}1.002 ± 0.050	^{a}0.040 ± 0.017
DMC	^{dc}0.205 ± 0.013	^{c}1.208 ± 0.030	^{a}0.064 ± 0.034
BDMC	^{c}0.282 ± 0.018	^{c}1.308 ± 0.071	^{a}0.071 ± 0.022
curcuminoids	^{ad}0.141 ± 0.037	^{d}0.987 ± 0.040	^{a}0.047 ± 0.011
THC	^{ac}0.075 ± 0.020	^{c}0.504 ± 0.047	^{a}0.041 ± 0.011
α-tocopherol	^{dc}0.218 ± 0.033	^{f}1.808 ± 0.120	^{a}-0.001 ± 0.006

Data were presented as mean ± S.E.M. of five independent experiments.

Means with the same letter are not significantly different.

Table 4.7 Effect of 10 μM curcuminoids, its derivatives and α-tocopherol on inhibition rate (R_{inh}) of TBARs formation in he-oxLDL.

Tested compounds	R_{inh} of TBARs formation (nmol/mg protein hr^{-1})		
	0-3 hr	3-9 hr	9-24 hr
curcumin	^{ab}0.471 ± 0.034	^{b}1.654 ± 0.148	^{ab}0.108 ± 0.066
DMC	^{b}0.374 ± 0.036	^{b}1.447 ± 0.158	^{b}0.122 ± 0.054
BDMC	^{b}0.297 ± 0.034	^{b}1.347 ± 0.181	^{b}-0.028 ± 0.053
curcuminoids	^{abd}0.438 ± 0.037	^{b}1.668 ± 0.179	^{abd}-0.005 ± 0.041
THC	^{a}0.504 ± 0.029	^{a}2.142 ± 0.189	^{a}0.001 ± 0.043
α-tocopherol	^{c}0.317 ± 0.071	^{c}0.847 ± 0.111	^{c}0.043 ± 0.026

Data were presented as mean ± S.E.M. of five independent experiments.

Means with the same letter are not significantly different.

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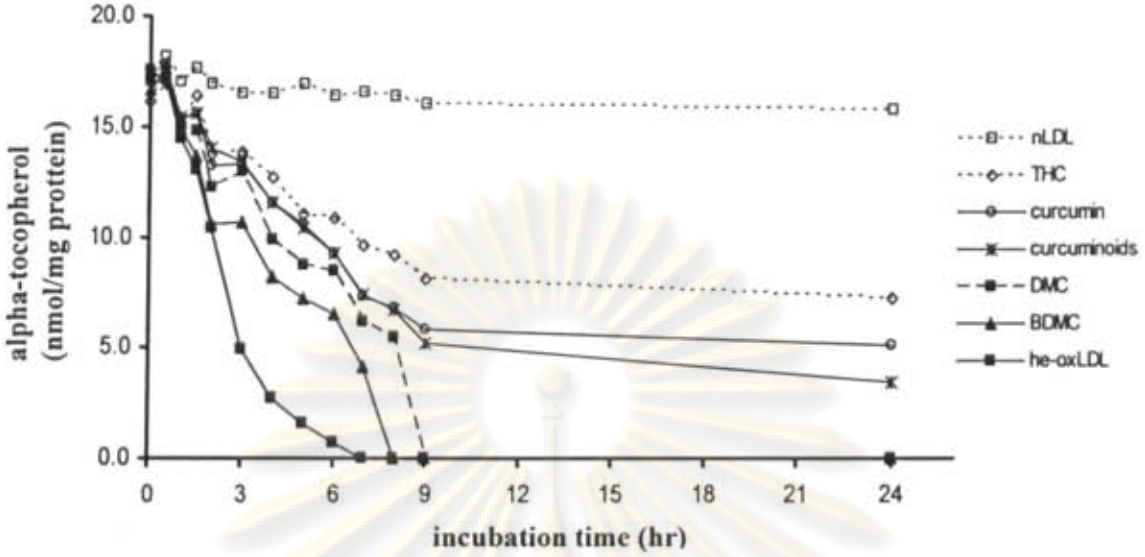

Figure 4.18 The time course effect of 10 μM curcumin, DMC, BDMC, curcuminoids and THC on levels of α-tocopherol in ox-LDL during incubated with hemin (n = 5).

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Table 4.8 Effect of 10 μM curcuminoids, its derivatives and α-tocopherol on decreasing rate of α-tocopherol in he-oxLDL.

condition	decreasing rate of α-tocopherol ($x10^{-2}$ nmol/mg protein min^{-1})		
	0-3 hr	3-9 hr	9-24 hr
nLDL	^{a}0.526 ± 0.114	^{a}0.058 ± 0.016	0
he-oxLDL	^{b}6.538 ± 0.330	^{b}3.382 ± 1.347*	0
curcumin	^{a}3.139 ± 0.617	^{a}0.588 ± 0.050	^{a}0.001 ± 0
DMC	^{a}3.535 ± 0.440	^{bc}2.571 ± 0.370	0
BDMC	^{ab}4.203 ± 0.340	^{c}3.137 ± 0.481**	0
curcuminoids	^{a}2.817 ± 0.327	^{ac}1.102 ± 0.383	^{a}0.002 ± 0
THC	^{a}1.366 ± 0.361	^{a}0.565 ± 0.027	^{a}0.001 ± 0
α-tocopherol	^{ab}7.008 ± 1.396	^{ab}1.483 ± 0.205	^{a}0.005 ± 0

Data were presented as mean ± S.E.M. of five independent experiments.

Means with the same letter are not significantly different.

*he-oxLDL : α-tocopherol decreased until undetectable at 5 hr of incubation

**BDMC : α-tocopherol decreased until undetectable at 8 hr of incubation

Table 4.9 Effect of 10 μM curcuminoids, its derivatives and α-tocopherol on inhibition rate (R_{inh}) of decreasing of α-tocopherol in he-oxLDL.

Tested compounds	R_{inh} of decreasing rate of α-tocopherol ($x\ 10^{-2}$ nmol/mg protein hr^{-1})	
	0-3 hr	3-9 hr
curcumin	^{ab}3.625 ± 0.968	^{a}5.005 ± 1.217
DMC	^{ab}3.003 ± 0.714	^{a}4.814 ± 1.451
BDMC	^{b}2.335 ± 0.639	^{ab}2.632 ± 0.042
curcuminoids	^{ab}3.721 ± 0.611	^{ab}2.803 ± 0.685
THC	^{a}5.642 ± 0.374	^{a}4.712 ± 1.199
α-tocopherol	^{c}-6.470 ± 1.193	^{b}-1.775 ± 0.126

Data were presented as mean ± S.E.M. of five independent experiments.

Means with the same letter are not significantly different.

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2. The effect of curcuminoids and its derivatives on cholesteryl esters.

The 10 μM curcuminoids, curcumin, DMC, BDMC, THC and α-tocopherol were able to protect the damaged of lipid in he-oxLDL along 24 hr. The results of this study show the time course effect of all tested compounds on percent damage of CA and CL (figure 4.19 A and B).

The protective effect on the damage of CA significantly decreasded in the order of THC ≥ curcuminoids ≥ curcumin > DMC ≥ BDMC ≥ α-tocopherol ($p<0.05$) while the percent damage of CL significantly decreasded in the order of THC ≥ curcuminoids = curcumin ≥ DMC = BDMC ≥ α-tocopherol ($p<0.05$).

In addition, all tested compounds were able to inhibit the decrease of CL/CO ratio in he-oxLDL along 24 hr of incubation (figure 4.20). We found that THC, curcumin and curcuminoids were significantly inhibited decrease of CL/CO ratio ($p<0.05$) and this ratio of these three compounds have a comparable levels with nLDL. While DMC, BDMC and α-tocopherol were not significantly inhibited decrease of CL/CO ratio ($p<0.05$).

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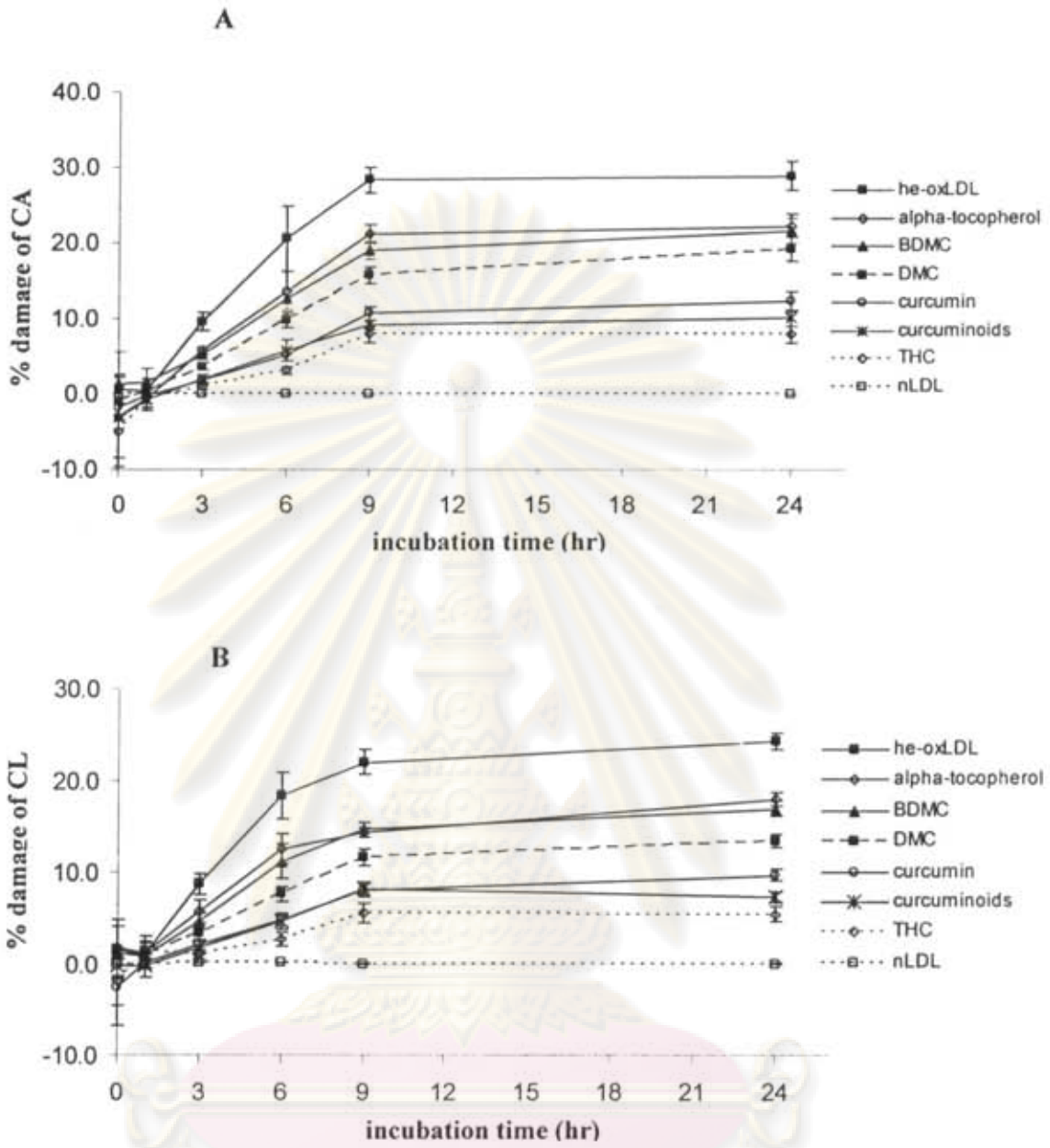

Figure 4.19 The time course effect of 10 μM curcuminoids, its derivatives and α-tocopherol on % damage of CA (A) and CL (B) in LDL during incubated with hemin (n = 5).

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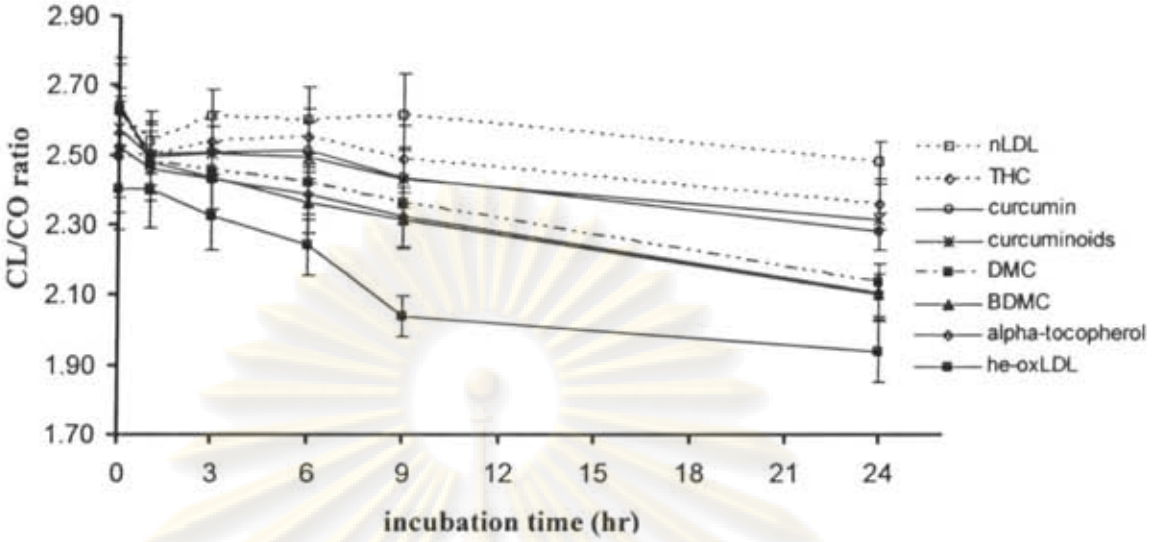

Figure 4.20 The time course effect of 10 μM curcuminoids, its derivatives and α-tocopherol on CL/CO ratio in LDL during incubated with hemin (n = 5).

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3. The effect of curcuminoids and its derivatives on oxidized lipid products.

The 10 μM curcuminoids, curcumin, DMC, BDMC, THC and α-tocopherol were able to inhibit the oxidized lipid products formation in he-oxLDL all retention time (9.6, 10.3, 11.6 and 12.3 min) along 24 hr of incubation (figure 4.21 A-D). We found that curcuminoids and its derivatives have more inhibitory effect on oxidized lipid products formation than α-tocopherol, a positive control.

In addition, curcuminoids and its derivatives almost completely inhibit oxidized lipid products formation at RT 10.3 and 11.6 min during 6-24 and 9-24 hr of incubation time, respectively (figure 4.21 B and C).

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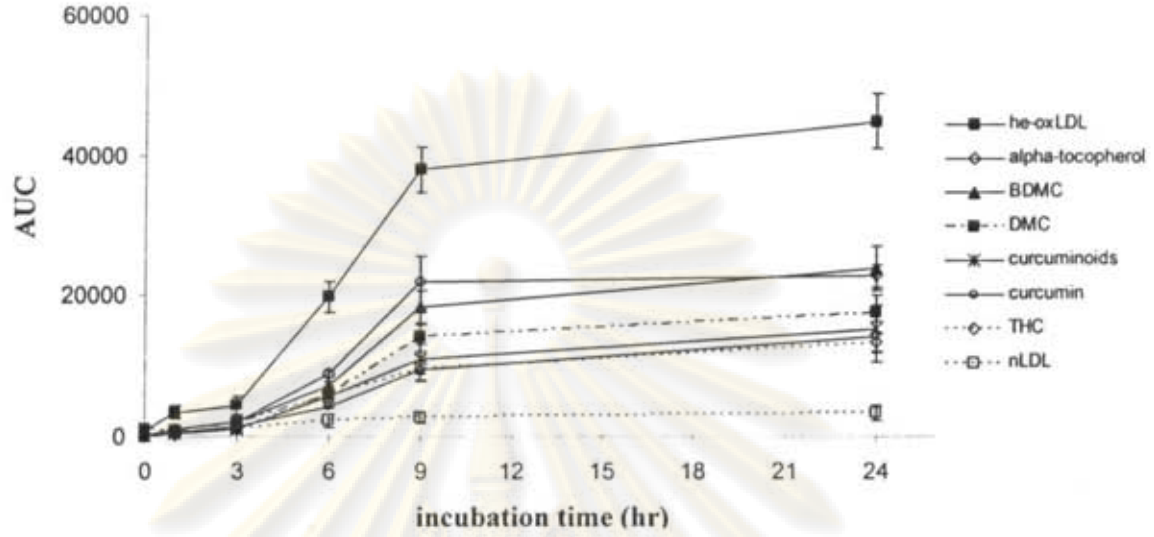
A oxidized lipid product at RT 9.6 min



B oxidized lipid product at RT 10.3 min

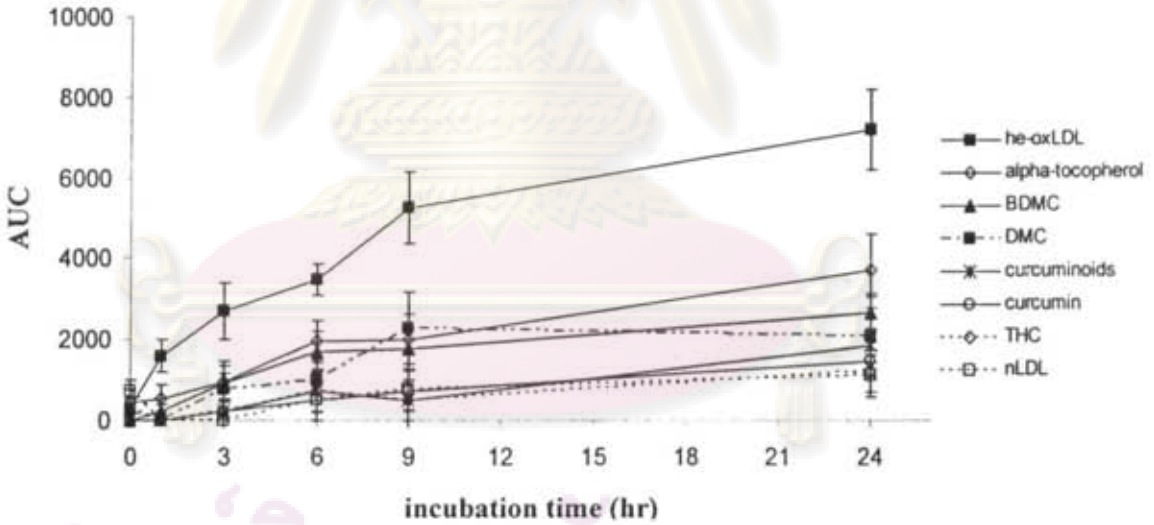


Figure 4.21 cont.

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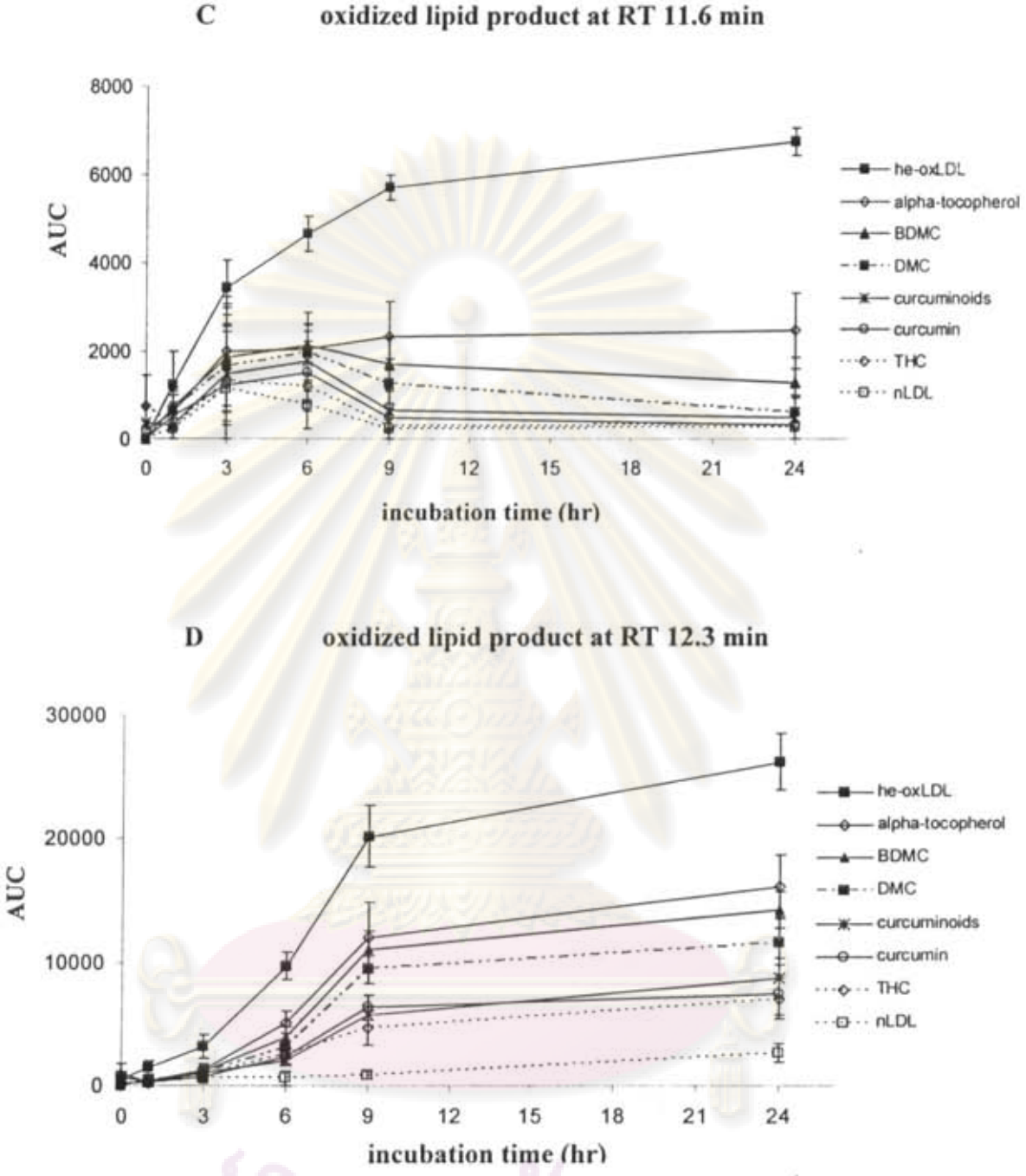

Figure 4.21 The time course effect of 10 μM curcuminoids, its derivatives and α-tocopherol on oxidized lipid products formation at retention time (RT) 9.6 min (A), 10.3 min (B), 11.6 min (C) and 12.3 min (D) (n = 5).

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CHAPTER V

DISCUSSION AND CONCLUSION

Our results were demonstrated that hemin induced LDL oxidation by increasing lipid peroxidation products as measured by TBARs formation. Moreover, the level of α-tocopherol, major lipid soluble endogenous antioxidant in LDL was decreased and disappeared until undetectable at 5 hr of incubation with hemin. α-Tocopherol show the inverse correlation with the level of TBARs (–0.935, $p<0.01$), especially at the lag phase of lipid peroxidation. When α-tocopherol was disappeared, TBARs formation was dramatically increased (log phase), indicating that α-tocopherol was able to protect the LDL from lipid peroxidation.

Hemin damaged lipid composition in the core of LDL, it can decrease the levels of cholesteryl esters, especially CA and CL. The percent decrease of CA and CL show a good correlation with TBARs ($r=0.857$, $p<0.01$ and 0.854, $p<0.01$ respectively), indicating that CA and CL are the major targets of lipid peroxidation induced by hemin. Since CA and CL are polyunsaturated fatty acid which susceptible to oxidation more than monounsaturated and saturated fatty acid. CL is the most abundant cholesteryl ester in LDL so it is a major target of lipid peroxidation (8). In addition, the oxidized lipid products were detectable in he-oxLDL. Our results were consistent with those reported by Vieira et al (73), oxidation of LDL promoted by ferrylmyoglobin induced a rapid consumption of both CA and CL, with turn into the corresponding hydroperoxides. Cholesteryl linoleate hydroperoxide (CL-OOH) increased in parallel with the loss of CL. So CL-OOH is a major product of LDL oxidation.

Furthermore, the CL/CO ratio of less than 2 has been suggested to be used as a clinical marker to determine the degree of clinical severity in β-thal/HbE patients (8). Our study has also shown that CL/CO ratio in he-oxLDL was decreased less than 2 (1.936 ± 0.086) at 24 hr of incubation. There was an inverse correlation between CL/CO ratio and TBARs level ($r=-0.682$, $p<0.01$), indicating that CL/CO ratio can be used as a marker of lipid damage in this study. Our *in vitro* LDL oxidation induced by hemin mimic the oxidative stress of LDL in thalassemia patients. The previous study found that there were high TBARs and corresponding low α-tocopherol levels as well

as the low levels of CEs and CL/CO ratio in both plasma and lipoprotein of β-thal/HbE patients, especially in moderately and severely affected patients (8). In addition, the amount of vitamin E in the β-thalassemic intermedia LDL was lower than healthy control (74).

The study of the effects of curcuminoids and its derivatives on he-oxLDL were found that all tested compounds possed an antioxidant activity, which protected hemin induced lipid peroxidation by inhibition of TBARs formation, the depletion of α-tocopherol, the damage of cholesteryl esters (CA and CL), the decrease of CL/CO ratio and the oxidized lipid products formation in dose dependent manner. The protective effect on oxidation parameters and lipid composition was in the order of THC ≥ curcumin ≥ curcuminoids > DMC > BDMC > α-tocopherol. We found similar effect on the ability of curcumin, DMC and BDMC to inhibit iron-stimulated lipid peroxidation in rat brain homogenate and rat liver microsomes, all compounds more potent than α-tocopherol (36).

The study of the time dependent effects of curcuminoids and its derivatives in the concentration of 10 μM on he-oxLDL found that the rate of TBARs formation and the decreasing rate of α-tocopherol in he-oxLDL have 3 phase. First phase was in 0-3 hr of incubation, TBARs levels were slighly increased while α-tocopherol was rapidly decreased indicating that α-tocopherol was consumed to protected LDL oxidation. Second phase was in 3-9 hr of incubation, α-tocopherol was rapidly decreased until undetectable at 5 hr and TBARs levels were rapidly increased up to reach the maximum levels in the third phase during 9-24 hr of incubation. Furthermore, when α-tocopherol were disappeared, the lipid especially CA and CL were dramatically damaged.

In addtion, THC, curcumin and curcuminoids were more potent to protect the damage of lipid than another tested compounds including α-tocopherol, a positive control. These three tested compounds were able to prolong the remainder of endogenous α-tocopherol in LDL oxidation throughout 24 hr of incubation and amount of the remainder depend on the concentration of tested compounds. While BDMC and DMC were able to prolong the remainder of endogenous α-tocopherol only 8 and 9 hr of incubation, respectively. So DMC and BDMC can protect the lipid damage less than another tested compounds.

There were some studies to compare the inhibitory effect of curcuminoids and its derivatives on lipid peroxidation. Curcumin was also found to be more effective than DMC and BDMC to inhibit lipid peroxidation in several models for example in the model of rat liver microsomes, erythrocyte membranes and brain homogenates. A comparative study on the antioxidant properties of THC and curcuminoids using the DPPH method, THC much more efficient than curcuminoids analogs (57). Inhibitory effect of curcumin and THC on the lipid peroxidation of erythrocyte membrane ghost induced by tert-butylhydroperoxide, THC showed a greater inhibitory effect than curcumin (55). Similarly, the *in vitro* effect of THC on the copper induced oxidation of LDL, THC is stronger than curcumin (56).

Since the lipid peroxidation is a free radical chain reaction and one initiating radical could induce up to fifty propagation reaction (75). Curcumin is a classical phenolic chain-breaking antioxidant, donating H-atom from the phenolic groups (76). In the study of Sirijaroonwong (77) also found that curcumin and its derivatives act as antioxidant by scavenging carbon-centered radicals, an intermediated radical in lipid peroxidation process rather than direct radical scavenger (hydroxyl radical and superoxide anion) that detected by electron spin resonance (ESR) spectroscopy. We suggested that curcumioids and its derivatives act as the chain breaking antioxidant by reacting with the chain propagating peroxyl radicals to inhibit lipid peroxidation induced by hemin.

The study of the antioxidant mechanism of curcumin by laser flash photolysis and pulse radiolysis in acidic and neutral aqueous and acetonotrile solution, curcumin is a superb H-atom donor by donating the H-atom from the central methylenic group rather than from the phenolic group (78). Wright (79) demonstrated that the bond dissociation enthalpy (BDE) of the phenolic O-H bond is significantly lower than the BDE of the C-H bond, suggesting that the H-atom abstraction shall take place in the phenolic group. Priyadasrini et al (80) have reported that curcumin show much greater ability to inhibit lipid peroxidation than its demethoxy counter part, suggesting that phenolic group is essential for antioxidant activity. De Heer et al (81) have demonstrated that antioxidant activity of curcumin is due to not only the number of phenolic group but also the high reactivity of ortho-methoxyphenolic and ortho-hydroxyphenolic funtionalities. The ortho-methoxy group can form intramolecular

hydrogen bond with the phenolic hydrogen, making the H-atom abstraction from the ortho-methoxy phenols surprisingly easy.

In our study demonstrated that THC was more potent to protect he-oxLDL than another tested compounds due to it has ortho-methoxyphenolic group while protective effect of DMC and BDMC were low because of the lack of ortho-methoxyphenolic group and less methoxy group than curcumin and THC.

According to the results of this study suggesting that the protective effect of curcuminoids and its derivatives on he-oxLDL can arise both from the ortho-methoxyphenolic group and from central methylenic hydrogen in the central seven carbon chain and β-diketone moiety. The decrease of one methoxy group of curcumin caused a great decrease in protective effect of curcumin.

In conclusion, THC, curcumin and curcuminoids were able to prolong the remainder of α-tocopherol levels throughout 24 hr of incubation. So they were able to protect lipid damaged while DMC, BDMC and α-tocopherol, a positive control can protect lipid damage in the higher concentration. We concluded that THC was more potent to protect he-oxLDL than another tested compounds follow by curcumin and curcuminoids that have a comparable effect and more potent than DMC, BDMC and α-tocopherol.

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APPENDICES

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APPENDIX A

The study of the concentration dependent effects of curcuminoids and its derivatives on hemin induced LDL oxidation (he-oxLDL).

1.The effect of curcuminoids, its derivatives and α-tocopherol on TBARs level.

At 24 hr of incubation time

Condition	Conc.	MDA (nmol/mg protein)					Mean	S.E.M
	(μM)	N 1	N 2	N 3	N 4	N 5		
nLDL	-	2.97	3.49	3.44	3.10	3.50	3.30	0.11
ox-LDL	-	14.21	14.48	15.91	15.48	14.84	14.98	0.31
curcumin	1	9.73	10.81	11.66	10.95	11.30	10.89	0.32
	2.5	8.53	9.54	10.55	10.13	9.45	9.64	0.34
	10	5.45	6.47	7.06	6.42	7.06	6.49	0.29
	50	3.93	4.72	4.77	4.32	4.41	4.43	0.15
	100	2.22	3.74	3.84	3.47	3.62	3.38	0.30
DMC	1	11.66	12.54	13.55	13.41	12.69	12.77	0.34
	2.5	9.62	10.45	11.02	10.82	10.96	10.58	0.26
	10	7.42	8.64	9.34	8.64	8.48	8.50	0.31
	50	5.94	6.82	7.22	6.58	6.34	6.58	0.22
	100	3.60	5.24	5.59	5.29	5.27	5.00	0.36
BDMC	1	12.44	12.87	14.60	14.20	13.76	13.57	0.40
	2.5	11.26	11.72	13.04	13.05	12.01	12.21	0.36
	10	9.00	9.99	11.17	10.47	10.38	10.20	0.36
	50	6.79	6.20	8.08	7.60	8.08	7.35	0.37
	100	4.69	5.57	6.32	5.93	6.35	5.77	0.31
curcuminoids	1	9.67	11.52	12.91	12.79	12.82	11.94	0.62
	2.5	8.41	10.55	11.54	11.42	10.96	10.58	0.57
	10	6.08	7.25	7.99	7.73	8.14	7.44	0.37
	50	4.60	5.70	6.42	6.23	6.09	5.81	0.32
	100	3.49	4.12	4.67	4.67	5.22	4.43	0.29
THC	1	10.00	9.07	10.06	10.30	8.97	9.68	0.27
	2.5	9.46	9.04	9.20	9.56	8.47	9.15	0.19
	10	7.40	7.03	7.16	7.34	7.13	7.21	0.07
	50	4.44	4.67	5.06	4.89	4.46	4.70	0.12
	100	3.26	4.02	4.20	4.33	3.80	3.92	0.19
α-tocopherol	10	10.33	10.15	12.20	12.39	11.18	11.25	0.46
	25	9.85	9.81	11.40	11.43	10.87	10.67	0.36
	50	7.90	7.75	9.71	9.76	8.31	8.69	0.44
	100	5.69	6.13	7.85	7.53	7.40	6.92	0.42
	500	2.65	3.20	3.97	4.23	3.79	3.57	0.28

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2. Effect of curcuminoids, its derivatives and α-tocopherol on % inhibition of TBARs. At 24 hr of incubation time

Tested compounds	Conc. (μM)	% inhibition of TBARs formation					Mean	S.E.M
		N 1	N 2	N 3	N 4	N 5		
curcumin	1	39.88	33.39	34.11	36.62	31.20	35.04	1.49
	2.5	50.61	45.00	42.97	43.19	47.53	45.86	1.44
	10	77.98	72.96	71.01	73.18	68.56	72.74	1.55
	50	91.50	88.88	89.37	90.14	91.96	90.37	0.59
	100	98.70	97.73	96.82	97.02	98.87	97.83	0.42
DMC	1	22.71	17.66	18.94	16.71	19.03	19.01	1.02
	2.5	40.84	36.70	39.19	37.63	34.19	37.71	1.13
	10	60.44	53.20	52.70	55.28	56.12	55.55	1.38
	50	73.60	69.69	69.67	71.91	74.96	71.97	1.05
	100	85.15	84.07	82.76	82.31	84.36	83.73	0.52
BDMC	1	15.81	9.92	10.53	10.33	9.55	11.23	1.16
	2.5	26.32	20.83	22.99	19.64	25.02	22.96	1.25
	10	46.40	37.16	38.00	40.48	39.33	40.27	1.63
	50	66.06	60.56	62.84	63.62	59.62	62.54	1.14
	100	74.50	79.11	76.90	77.14	74.81	76.49	0.84
curcuminoids	1	25.72	22.70	24.07	21.68	17.87	22.41	1.32
	2.5	38.08	31.92	35.09	32.74	34.23	34.41	1.07
	10	60.87	63.12	63.55	62.59	59.03	61.83	0.84
	50	75.36	77.89	76.12	74.71	77.17	76.25	0.58
	100	86.27	92.86	90.15	87.32	84.77	88.27	1.44
THC	1	40.76	45.90	44.28	43.77	46.00	44.14	0.95
	2.5	45.06	46.27	51.17	49.46	50.64	48.52	1.21
	10	61.50	65.27	67.54	66.66	63.20	64.83	1.11
	50	85.45	87.60	84.38	85.62	88.25	86.26	0.72
	100	88.51	93.78	91.24	89.97	94.40	91.58	1.12
α-tocopherol	10	19.24	26.93	27.16	27.55	25.25	25.23	1.55
	25	23.94	30.11	33.56	34.99	28.21	30.16	1.97
	100	43.02	49.34	47.13	47.96	52.14	47.92	1.49
	250	64.66	64.47	62.03	65.21	60.65	63.40	0.88
	500	94.48	91.80	93.05	90.77	94.50	92.92	0.74

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3. IC_{50} values of curcuminoids, its derivatives and α-tocopherol on TBARs formation.

At 24 hr of incubation time.

Tested compounds	IC_{50} (μM)					Mean	S.E.M
	N 1	N 2	N 3	N 4	N 5		
curcumin	1.97	3.02	3.17	2.84	3.17	2.83	0.22
DMC	6.26	8.83	8.50	8.51	8.01	8.02	0.46
BDMC	14.46	18.79	18.13	17.83	19.49	17.74	0.87
curcuminoids	5.96	6.30	6.02	6.93	7.60	6.56	0.31
THC	2.96	2.20	1.92	2.10	1.98	2.23	0.19
α-tocopherol	83.36	67.53	66.92	63.62	69.54	70.19	3.43

4. IC_{50} values of curcuminoids, its derivatives and α-tocopherol on decreasing of CA.

At 24 hr of incubation time.

Tested compounds	IC_{50} (μM)					Mean	S.E.M
	N1	N2	N3	N4	N5		
curcumin	8.15	9.44	9.99	8.94	8.93	9.09	0.06
DMC	21.08	23.24	26.93	23.70	26.08	24.21	0.78
BDMC	41.14	44.72	42.37	39.60	32.18	40.00	0.30
curcuminoids	7.85	10.63	11.33	9.19	8.89	9.58	0.26
THC	6.62	4.90	5.02	4.73	4.92	5.24	0.29
α-tocopherol	78.78	65.82	69.08	70.98	70.42	71.02	0.25

5. IC_{50} values of curcuminoids, its derivatives and α-tocopherol on decreasing of CL.

At 24 hr of incubation time.

Tested compounds	IC_{50} (μM)					Mean	S.E.M
	N1	N2	N3	N4	N5		
curcumin	8.15	6.41	5.95	6.47	6.45	6.69	0.34
DMC	21.08	15.48	15.18	15.17	13.00	15.98	1.21
BDMC	41.14	35.84	37.91	34.13	39.77	37.76	1.14
curcuminoids	7.85	10.50	11.83	8.97	7.95	9.42	0.69
THC	6.62	6.07	7.12	5.78	5.26	6.17	0.29
α-tocopherol	78.78	49.4	59.73	52.42	47.18	57.50	5.12

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6. IC_{50} values of curcuminoids, its derivatives and α-tocopherol on decreasing of CL/CO ratio.

At 24 hr of incubation time.

Tested compounds	CL/CO ratio (μmol/mg protein)					Mean	S.E.M
	N1	N2	N3	N4	N5		
curcumin	5.94	8.18	8.18	8.08	9.72	8.02	0.60
DMC	17.68	14.82	16.75	18.3	25.29	18.57	1.78
BDMC	28.35	23.61	34.73	22.08	55.22	32.80	6.02
curcuminoids	9.33	8.16	10.83	11.97	17.84	11.63	1.68
THC	3.92	5.85	7.87	5.68	2.52	5.17	0.91
α-tocopherol	72.34	29.59	93.49	61.47	39.02	59.18	11.48

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7. The effect of curcuminoids, its derivatives and α-tocopherol on α-tocopherol levels. At 24 hr of incubation time

Condition	Conc.	α-tocopherol (nmol/mg protein)					Mean	S.E.M
	(μM)	N 1	N 2	N 3	N 4	N 5		
nLDL	-	20.50	16.06	15.24	16.06	15.54	16.68	1.16
he-oxLDL	-	0	0	0	0	0	0	0
curcumin	1	0	0	0	0	0	0	0
	2.5	0	0	0	0	0	0	0
	10	5.31	3.55	2.88	3.75	4.02	3.90	0.48
	50	6.48	4.83	4.89	5.15	4.76	5.22	0.39
	100	9.72	6.80	6.48	6.58	5.87	7.09	0.44
DMC	1	0	0	0	0	0	0	0
	2.5	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0
	50	0	0	0	0	0	0	0
	100	8.65	4.99	4.32	4.69	4.18	5.36	0.66
BDMC	1	0	0	0	0	0	0	0
	2.5	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0
	50	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0
curcuminoids	1	0	0	0	0	0	0	0
	2.5	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0
	50	0	5.00	4.31	5.21	4.90	3.89	1.18
	100	0	7.24	4.94	5.38	5.52	4.61	1.47
THC	1	0	0	0	0	0	0	0
	2.5	0	0	0	0	0	0	0
	10	3.73	4.54	4.12	4.12	3.95	4.10	0.16
	50	4.94	5.62	5.23	4.86	5.00	5.13	0.16
	100	9.19	8.88	7.38	7.82	8.27	8.31	0.32
α-tocopherol	10	0	0	0	0	0	0	0
	25	0	0	0	0	0	0	0
	100	164.4	144.8	128.3	135.8	111.6	137.0	6.8
	250	319.9	576.8	497.4	534.6	467.3	479.2	64.8
	500	685.1	1327.8	1380.9	1310.8	1208.1	1182.5	180.6

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8. The effect of curcuminoids , its derivatives and α-tocopherol on % remainder of α-tocopherol.

At 24 hr of incubation time.

Tested compounds	Conc. (μM)	% remainder of α-tocopherol					Mean	S.E.M
		N 1	N 2	N 3	N 4	N 5		
curcumin	1	0	0	0	0	0	0	0
	2.5	0	0	0	0	0	0	0
	10	21.54	22.10	18.87	23.36	25.86	22.35	1.32
	50	26.27	30.05	32.07	32.07	30.62	30.21	0.42
	100	40.17	42.31	42.51	40.97	37.76	40.74	0.85
DMC	1	0	0	0	0	0	0	0
	2.5	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0
	50	0	0	0	0	0	0	0
	100	35.74	31.06	28.33	29.18	26.89	30.24	1.53
BDMC	1	0	0	0	0	0	0	0
	2.5	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0
	50	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0
curcuminoids	1	0	0	0	0	0	0	0
	2.5	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0
	50	0	28.70	28.25	32.43	31.67	24.21	6.05
	100	0	41.54	32.44	33.48	35.42	28.58	7.10
THC	1	0	0	0	0	0	0	0
	2.5	0	0	0	0	0	0	0
	10	16.82	26.08	28.06	26.66	25.73	24.67	0.84
	50	22.25	32.25	35.55	31.42	32.54	30.80	1.48
	100	41.41	50.99	50.21	50.57	53.84	49.40	2.08
α-tocopherol	10	0	0	0	0	0	0	0
	25	0	0	0	0	0	0	0
	100	30.00	29.97	27.09	32.30	25.95	29.06	1.13
	250	24.12	49.52	43.57	45.02	41.55	40.76	4.37
	500	29.56	63.83	59.87	56.73	54.39	52.87	6.04

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9. The effect of curcuminoids and its derivatives on cholesteryl esters.

Free cholesterol (FC) at 24 hr of incubation time

Condition	Conc. (μM)	FC (μmol/mg protein)					Mean	S.E.M
		N 1	N 2	N 3	N 4	N 5		
nLDL	-	2.234	2.514	2.094	2.118	2.055	2.203	0.083
he-oxLDL	-	2.346	2.478	2.125	2.195	1.927	2.214	0.094
curcumin	1	2.463	2.357	2.095	2.128	2.095	2.228	0.077
	2.5	2.447	2.486	2.368	2.246	2.015	2.312	0.085
	10	2.316	2.497	2.286	2.066	1.921	2.217	0.101
	50	2.337	2.386	2.219	2.420	2.227	2.318	0.041
	100	2.309	2.471	2.067	2.280	2.055	2.236	0.079
DMC	1	2.324	2.945	2.306	2.071	1.918	2.313	0.175
	2.5	2.675	2.626	2.065	2.487	1.922	2.355	0.152
	10	2.536	2.464	2.074	2.108	1.907	2.218	0.121
	50	2.711	2.510	2.105	2.178	2.070	2.315	0.126
	100	2.581	2.428	2.309	2.249	2.118	2.337	0.079
BDMC	1	2.516	2.227	2.246	2.220	1.919	2.226	0.095
	2.5	2.507	2.365	2.320	2.240	2.178	2.322	0.056
	10	2.583	2.378	2.326	2.371	2.003	2.332	0.094
	50	2.442	2.253	2.142	2.126	2.116	2.216	0.062
	100	2.321	2.200	2.269	2.015	2.181	2.197	0.052
curcuminoids	1	2.611	2.066	2.232	2.405	1.871	2.237	0.129
	2.5	2.556	2.227	2.150	2.107	1.923	2.193	0.104
	10	2.489	2.329	2.251	2.056	2.150	2.255	0.074
	50	2.656	2.201	2.322	2.107	2.090	2.275	0.104
	100	2.467	2.168	2.205	2.364	2.181	2.277	0.059
THC	1	2.619	2.227	2.208	2.082	2.280	2.283	0.090
	2.5	2.691	2.417	2.292	2.189	2.092	2.336	0.104
	10	2.977	2.356	2.398	2.122	2.220	2.415	0.149
	50	2.441	2.288	2.470	1.979	2.173	2.270	0.090
	100	2.485	2.227	2.147	2.308	2.289	2.291	0.056
α-tocopherol	10	2.541	2.103	2.298	2.257	2.012	2.242	0.091
	25	2.421	2.182	2.282	2.073	2.027	2.197	0.071
	100	2.514	2.135	2.290	2.189	2.173	2.260	0.068
	250	2.528	2.048	2.108	2.363	2.214	2.252	0.087
	500	2.433	2.017	2.273	2.027	2.283	2.207	0.081

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Cholesteryl arachidonate (CA) at 24 hr of incubation time

Condition	Conc. (μM)	CA (μmol/mg protein)					Mean	S.E.M
		N 1	N 2	N 3	N 4	N 5		
nLDL	-	0.493	0.514	0.469	0.489	0.484	0.490	0.007
he-oxLDL	-	0.366	0.395	0.360	0.382	0.367	0.374	0.006
curcumin	1	0.379	0.410	0.371	0.398	0.378	0.387	0.007
	2.5	0.402	0.427	0.388	0.413	0.396	0.405	0.007
	10	0.441	0.457	0.413	0.440	0.425	0.435	0.007
	50	0.466	0.482	0.441	0.460	0.456	0.461	0.007
	100	0.582	0.502	0.460	0.481	0.480	0.501	0.021
DMC	1	0.375	0.407	0.367	0.395	0.379	0.385	0.007
	2.5	0.394	0.419	0.380	0.409	0.396	0.400	0.007
	10	0.398	0.432	0.390	0.417	0.403	0.408	0.007
	50	0.449	0.465	0.421	0.441	0.426	0.440	0.008
	100	0.551	0.486	0.441	0.459	0.462	0.480	0.019
BDMC	1	0.367	0.389	0.365	0.388	0.370	0.376	0.005
	2.5	0.380	0.398	0.374	0.400	0.385	0.387	0.005
	10	0.387	0.405	0.387	0.414	0.394	0.397	0.005
	50	0.437	0.431	0.417	0.437	0.432	0.431	0.004
	100	0.535	0.440	0.428	0.448	0.439	0.458	0.020
curcuminoids	1	0.456	0.393	0.370	0.397	0.379	0.399	0.015
	2.5	0.481	0.407	0.388	0.414	0.395	0.417	0.017
	10	0.536	0.439	0.419	0.447	0.431	0.454	0.021
	50	0.551	0.448	0.437	0.461	0.448	0.469	0.021
	100	0.568	0.465	0.451	0.469	0.462	0.483	0.021
THC	1	0.400	0.406	0.417	0.401	0.386	0.402	0.005
	2.5	0.443	0.427	0.442	0.433	0.411	0.431	0.006
	10	0.488	0.444	0.460	0.441	0.427	0.452	0.010
	50	0.503	0.454	0.478	0.454	0.437	0.465	0.011
	100	0.579	0.472	0.499	0.471	0.448	0.494	0.023
α-tocopherol	10	0.453	0.398	0.406	0.394	0.387	0.408	0.012
	25	0.464	0.404	0.417	0.401	0.394	0.416	0.013
	100	0.522	0.447	0.457	0.441	0.422	0.458	0.017
	250	0.548	0.467	0.480	0.457	0.436	0.478	0.019
	500	0.570	0.481	0.492	0.468	0.453	0.493	0.020

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Cholesteryl linoleate (CL) at 24 hr of incubation time

Condition	Conc. (μM)	CL (μmol/mg protein)					mean	S.E.M
		N 1	N 2	N 3	N 4	N 5		
nLDL	-	2.113	2.125	2.219	2.236	1.996	2.138	0.043
he-oxLDL	-	1.687	1.697	1.795	1.843	1.614	1.727	0.041
curcumin	1	1.836	1.812	1.921	1.951	1.704	1.845	0.044
	2.5	1.896	1.855	1.954	1.972	1.740	1.883	0.041
	10	1.932	1.882	1.989	2.037	1.780	1.924	0.044
	50	2.118	2.064	2.141	2.174	1.906	2.081	0.047
	100	2.095	2.102	2.200	2.205	1.958	2.112	0.045
DMC	1	1.751	1.756	1.843	1.892	1.654	1.779	0.041
	2.5	1.805	1.824	1.913	1.948	1.725	1.843	0.040
	10	1.866	1.863	1.974	2.015	1.757	1.895	0.046
	50	1.989	1.964	2.056	2.091	1.863	1.993	0.040
	100	1.996	2.037	2.138	2.151	1.887	2.042	0.049
BDMC	1	1.734	1.752	1.846	1.907	1.664	1.781	0.043
	2.5	1.778	1.778	1.856	1.921	1.704	1.807	0.037
	10	1.815	1.836	1.925	1.988	1.743	1.861	0.043
	50	1.953	1.912	2.030	2.054	1.805	1.951	0.045
	100	1.948	1.969	2.080	2.126	1.902	2.005	0.042
curcuminoids	1	1.751	1.786	1.865	1.922	1.677	1.800	0.043
	2.5	1.817	1.827	1.923	1.979	1.755	1.860	0.040
	10	1.865	1.897	1.991	2.044	1.821	1.924	0.041
	50	1.915	1.938	2.051	2.098	1.858	1.972	0.044
	100	2.032	2.026	2.168	2.195	1.947	2.074	0.047
THC	1	1.764	1.796	1.900	1.939	1.916	1.863	0.035
	2.5	1.822	1.850	1.968	1.984	1.935	1.912	0.032
	10	1.859	1.943	2.047	2.053	2.039	1.988	0.038
	50	2.063	1.991	2.139	2.127	2.086	2.081	0.026
	100	2.055	2.056	2.206	2.161	2.143	2.124	0.030
α-tocopherol	10	1.786	1.946	1.912	1.938	1.895	1.895	0.029
	25	1.821	1.998	1.967	1.967	1.920	1.935	0.031
	100	1.852	2.201	2.009	2.018	1.992	2.014	0.056
	250	2.043	2.167	2.161	2.150	2.073	2.119	0.025
	500	2.099	2.219	2.217	2.188	2.126	2.170	0.024

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Cholesteryl oleate (CO) at 24 hr of incubation time

Condition	Conc (μM)	CO (μmol/mg protein)					Mean	S.E.M
		N 1	N 2	N 3	N 4	N 5		
nLDL	-	0.798	0.844	0.860	0.870	0.818	0.838	0.013
he-oxLDL	-	0.783	0.835	0.887	0.861	0.852	0.844	0.017
curcumin	1	0.788	0.843	0.891	0.870	0.860	0.850	0.017
	2.5	0.793	0.835	0.883	0.865	0.837	0.843	0.015
	10	0.792	0.838	0.871	0.871	0.845	0.843	0.014
	50	0.810	0.852	0.889	0.876	0.836	0.853	0.014
	100	0.737	0.855	0.867	0.878	0.833	0.834	0.025
DMC	1	0.773	0.834	0.847	0.866	0.833	0.831	0.016
	2.5	0.795	0.831	0.853	0.853	0.841	0.835	0.011
	10	0.788	0.839	0.896	0.885	0.848	0.851	0.019
	50	0.806	0.833	0.889	0.883	0.842	0.851	0.016
	100	0.741	0.837	0.871	0.859	0.838	0.829	0.023
BDMC	1	0.798	0.795	0.892	0.878	0.854	0.843	0.020
	2.5	0.790	0.792	0.888	0.861	0.838	0.834	0.019
	10	0.762	0.784	0.833	0.853	0.831	0.813	0.017
	50	0.808	0.788	0.890	0.855	0.844	0.837	0.018
	100	0.730	0.800	0.879	0.874	0.860	0.829	0.028
curcuminoids	1	0.759	0.791	0.892	0.861	0.837	0.828	0.024
	2.5	0.730	0.782	0.896	0.874	0.829	0.822	0.030
	10	0.725	0.788	0.889	0.882	0.826	0.822	0.031
	50	0.729	0.788	0.828	0.876	0.831	0.810	0.025
	100	0.727	0.807	0.855	0.865	0.838	0.818	0.025
THC	1	0.791	0.788	0.846	0.840	0.868	0.827	0.016
	2.5	0.789	0.788	0.810	0.827	0.824	0.808	0.008
	10	0.799	0.802	0.836	0.844	0.834	0.823	0.009
	50	0.793	0.810	0.846	0.833	0.827	0.822	0.009
	100	0.721	0.804	0.841	0.830	0.829	0.805	0.022
α-tocopherol	10	0.767	0.859	0.836	0.844	0.841	0.829	0.016
	25	0.753	0.825	0.846	0.838	0.837	0.820	0.017
	100	0.742	0.818	0.848	0.837	0.830	0.815	0.019
	250	0.741	0.846	0.843	0.845	0.826	0.820	0.020
	500	0.750	0.849	0.855	0.838	0.824	0.823	0.019

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Cholesteryl palmitate (CP) at 24 hr of incubation time

Condition	Conc. (μM)	CP (μmol/mg protein) N 1	N 2	N 3	N 4	N 5	Mean	S.E.M
nLDL	-	0.591	0.560	0.602	0.549	0.658	0.592	0.019
he-oxLDL	-	0.475	0.577	0.586	0.535	0.639	0.562	0.027
curcumin	1	0.633	0.625	0.581	0.570	0.624	0.607	0.013
	2.5	0.676	0.549	0.558	0.501	0.638	0.584	0.032
	10	0.639	0.593	0.603	0.564	0.662	0.612	0.017
	50	0.652	0.569	0.632	0.581	0.626	0.612	0.016
	100	0.598	0.629	0.515	0.570	0.607	0.584	0.020
DMC	1	0.480	0.540	0.672	0.581	0.662	0.587	0.036
	2.5	0.548	0.631	0.578	0.437	0.578	0.554	0.032
	10	0.501	0.584	0.705	0.502	0.632	0.585	0.039
	50	0.616	0.548	0.667	0.601	0.657	0.618	0.021
	100	0.551	0.579	0.696	0.502	0.631	0.592	0.033
BDMC	1	0.547	0.538	0.654	0.598	0.622	0.592	0.022
	2.5	0.638	0.523	0.578	0.564	0.584	0.577	0.019
	10	0.672	0.502	0.623	0.579	0.661	0.607	0.031
	50	0.539	0.593	0.677	0.563	0.668	0.608	0.028
	100	0.526	0.526	0.629	0.633	0.577	0.578	0.023
curcuminoids	1	0.625	0.506	0.678	0.581	0.681	0.614	0.033
	2.5	0.577	0.493	0.632	0.502	0.666	0.574	0.034
	10	0.511	0.526	0.563	0.552	0.677	0.566	0.029
	50	0.570	0.593	0.599	0.495	0.611	0.574	0.021
	100	0.509	0.508	0.517	0.502	0.618	0.531	0.022
THC	1	0.495	0.539	0.503	0.636	0.601	0.555	0.028
	2.5	0.674	0.506	0.624	0.689	0.632	0.625	0.032
	10	0.616	0.483	0.565	0.640	0.603	0.581	0.027
	50	0.559	0.593	0.543	0.593	0.630	0.584	0.015
	100	0.613	0.524	0.555	0.604	0.598	0.579	0.017
α-tocopherol	10	0.571	0.593	0.524	0.639	0.649	0.595	0.023
	25	0.522	0.523	0.554	0.551	0.598	0.550	0.014
	100	0.584	0.506	0.661	0.504	0.633	0.578	0.032
	250	0.583	0.484	0.639	0.583	0.613	0.580	0.026
	500	0.508	0.501	0.583	0.631	0.642	0.573	0.030

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10. The effect of curcuminoids, its derivatives and α-tocopherol on CL/CO ratio.

At 24 hr of incubation time

Condition	Conc.	CL/CO ratio					Mean	S.E.M.
	(μM)	N1	N2	N3	N4	N5		
nLDL	-	2.647	2.517	2.579	2.571	2.441	2.551	0.034
he-oxLDL	-	2.077	2.034	2.024	2.141	1.894	2.034	0.041
curcumin	1	2.240	2.149	2.157	2.242	1.982	2.154	0.047
	2.5	2.298	2.221	2.214	2.281	2.108	2.224	0.033
	10	2.347	2.246	2.284	2.339	2.136	2.270	0.038
	50	2.547	2.422	2.409	2.483	2.253	2.423	0.049
	100	2.842	2.458	2.538	2.512	2.324	2.535	0.085
DMC	1	2.209	2.105	2.175	2.185	1.966	2.128	0.044
	2.5	2.253	2.196	2.244	2.283	2.033	2.202	0.045
	10	2.287	2.222	2.202	2.275	2.038	2.205	0.045
	50	2.422	2.358	2.314	2.369	2.227	2.338	0.033
	100	2.694	2.435	2.455	2.505	2.272	2.472	0.068
BDMC	1	2.127	2.204	2.069	2.172	1.949	2.104	0.045
	2.5	2.187	2.245	2.110	2.232	2.000	2.155	0.045
	10	2.332	2.342	2.311	2.331	2.096	2.283	0.047
	50	2.359	2.427	2.280	2.402	2.139	2.321	0.052
	100	2.670	2.460	2.366	2.432	2.210	2.428	0.074
curcuminoids	1	2.307	2.259	2.090	2.225	2.014	2.179	0.055
	2.5	2.490	2.336	2.146	2.263	2.086	2.264	0.071
	10	2.572	2.406	2.240	2.317	2.133	2.334	0.075
	50	2.627	2.459	2.477	2.396	2.126	2.417	0.082
	100	2.796	2.511	2.537	2.536	2.310	2.538	0.077
THC	1	2.301	2.280	2.246	2.307	2.208	2.268	0.019
	2.5	2.378	2.347	2.428	2.399	2.265	2.363	0.028
	10	2.471	2.421	2.482	2.431	2.474	2.456	0.012
	50	2.607	2.457	2.529	2.553	2.571	2.543	0.025
	100	2.849	2.558	2.622	2.603	2.707	2.668	0.051
α-tocopherol	10	2.329	2.267	2.289	2.297	2.331	2.302	0.012
	50	2.418	2.409	2.326	2.348	2.333	2.367	0.019
	100	2.497	2.469	2.370	2.411	2.496	2.449	0.025
	250	2.757	2.563	2.565	2.545	2.426	2.571	0.053
	500	2.797	2.614	2.593	2.610	2.579	2.639	0.040

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11. The effect of curcuminoids, its derivatives and α-tocopherol on oxidized lipid products formation.

RT 9.6 min at 24 hr of incubation time

Condition	Conc. (μM)	AUC of oxidized lipid product at RT 9.6 min					Mean	S.E.M.
		N1	N2	N3	N4	N5		
nLDL	-	0	1521	0	1542	1632	939	384
he-oxLDL	-	15612	19789	32243	25478	24267	23478	2801
curcumin	1	8809	12781	19963	17758	16527	15168	1971
	2.5	7976	9932	15563	14479	13257	12241	1425
	10	5055	8083	11096	10563	10852	9130	1153
	50	3733	6610	9633	7589	8562	7225	1007
	100	2509	3518	4965	3654	5632	4056	555
DMC	1	10043	14103	21665	18523	16113	16089	1969
	2.5	8116	12082	19563	15450	14521	13946	1892
	10	6604	9818	14365	12443	12302	11106	1337
	50	6886	9112	12556	10556	8965	9615	940
	100	5669	7527	9553	7855	6587	7438	652
BDMC	1	12321	18136	27125	21453	20323	19872	2402
	2.5	10998	16295	23633	20041	18254	17844	2095
	10	8541	12352	18456	16336	15632	14263	1734
	50	6961	10256	15966	13992	12301	11895	1552
	100	7652	9758	14563	13369	9625	10993	1284
curcuminoids	1	13201	18695	28596	21563	20365	20484	2482
	2.5	10659	15632	23632	19965	18521	17682	2178
	10	8562	13126	18456	16587	15231	14392	1698
	50	5632	9236	12896	10047	8659	9294	1170
	100	3251	4965	8526	7413	6521	6135	927
THC	1	10234	16125	1546	1650	1723	6256	2976
	2.5	9234	14665	34665	24733	22840	21227	4373
	10	6643	10268	26125	18965	17254	15851	3415
	50	4521	6365	24566	15536	14332	13064	3591
	100	2158	3658	16563	13258	10254	9178	2758
α-tocopherol	10	13950	16063	31452	21644	19325	20487	3043
	50	13559	14965	28859	19256	17521	18832	2694
	100	8563	10236	22632	16325	13254	14202	2490
	250	6179	7253	14965	11555	11259	10242	1589
	500	5789	6065	12263	9669	7745	8306	1208

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RT 10.3 min at 24 hr of incubation time

Condition	Conc. (μM)	AUC of oxidized lipid product at RT 10.3 min					Mean	S.E.M.
		N1	N2	N3	N4	N5		
nLDL	-	0	0	0	0	0	0	0
he-oxLDL	-	1542	1242	4758	3985	3365	2978	686
curcumin	1	0	0	1009	0	0	202	202
	2.5	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0
	50	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0
DMC	1	0	0	896	1125	1254	655	273
	2.5	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0
	50	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0
BDMC	1	1253	1763	2563	2546	2214	2068	250
	2.5	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0
	50	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0
curcuminoids	1	1229	1245	1114	1063	100	950	215
	2.5	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0
	50	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0
THC	1	10234	1785	0	0	0	2404	1988
	2.5	9234	0	5014	4126	3852	4445	1474
	10	6643	0	0	0	0	1329	1329
	50	4521	0	0	0	0	904	904
	100	0	0	0	0	0	0	0
α-tocopherol	10	1035	1451	1227	1125	1564	1280	99
	50	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0
	250	0	0	0	0	0	0	0
	500	0	0	0	0	0	0	0

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RT 11.6 min at 24 hr of incubation time

Condition	Conc. (μM)	AUC of oxidized lipid product at RT 11.6 min					Mean	S.E.M.
		N1	N2	N3	N4	N5		
nLDL	-	0	0	0	0	0	0	0
he-oxLDL	-	2507	9655	7003	4966	4187	5664	1232
curcumin	1	0	909	0	0	0	182	182
	2.5	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0
	50	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0
DMC	1	1423	2689	2314	1489	1454	1874	263
	2.5	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0
	50	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0
BDMC	1	1547	2865	3162	2589	2816	2596	278
	2.5	1865	0	0	0	0	373	373
	10	0	0	0	0	0	0	0
	50	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0
curcuminoids	1	1229	2463	2252	1345	1189	1696	273
	2.5	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0
	50	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0
THC	1	1125	0	0	0	0	225	225
	2.5	0	0	7362	5004	4562	3386	1462
	10	0	0	0	0	0	0	0
	50	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0
α-tocopherol	10	1688	2489	2489	2113	2562	2268	165
	50	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0
	250	0	0	0	0	0	0	0
	500	0	0	0	0	0	0	0

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RT 12.3 min at 24 hr of incubation time

Condition	Conc. (μM)	AUC of oxidized lipid product at RT 12.3 min					Mean	S.E.M.
		N1	N2	N3	N4	N5		
nLDL	-	2831	2539	1450	1450	1450	1944	306
he-oxLDL	-	33425	14029	29863	21470	19665	23690	3517
curcumin	1	17669	8732	17563	12541	10412	13383	1830
	2.5	14532	7200	14145	9446	7563	10577	1583
	10	10801	5356	9963	6444	5285	7570	1174
	50	6106	4138	6563	5556	4254	5323	487
	100	2965	3018	4012	3522	2263	3156	293
DMC	1	29363	12103	25632	17590	15363	20010	3233
	2.5	15217	7152	13563	11123	11215	11654	1362
	10	16030	6822	11142	8965	7525	10097	1657
	50	9246	5618	8965	7244	5321	7279	816
	100	5252	3889	5426	4623	5014	4841	273
BDMC	1	30125	15263	26965	19654	17325	21866	2858
	2.5	22016	12065	20456	15524	13211	16654	1967
	10	19854	10236	18156	14226	11214	14737	1882
	50	12788	6658	11568	9547	8566	9825	1084
	100	8907	4256	7563	6258	6698	6736	767
curcuminoids	1	21328	11256	20632	15458	12532	16241	2054
	2.5	18568	9656	17456	12522	10252	13691	1836
	10	13421	7658	14965	11400	8556	11200	1391
	50	10235	6169	11588	8966	6632	8718	1036
	100	4606	2669	6156	5522	5814	4953	626
THC	1	14231	9965	1270	0	0	5093	2947
	2.5	7787	6236	31452	20473	17563	16702	4591
	10	4715	3856	18565	10523	8856	9303	2629
	50	4521	2596	12632	8526	7525	7160	1728
	100	5204	1985	9365	5022	4932	5302	1178
α-tocopherol	10	23866	12826	22655	14252	13254	17371	2423
	50	20668	11536	20456	12455	10219	15067	2272
	100	16865	10663	18453	11450	8964	13279	1850
	250	12521	6965	12965	8966	5960	9475	1421
	500	8665	5359	9256	5787	4183	6650	984

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12. The effect of curcuminoids, its derivatives and α-tocopherol on total cholesterol levels.

At 24 hr of incubation time

Condition	Conc. (μM)	Total cholesterol (μmol/mg protein) N 1	N 2	N 3	N 4	N 5	Mean	S.E.M.
nLDL	-	7.90	7.18	7.46	6.66	6.86	7.21	0.22
he-oxLDL	-	6.87	6.99	7.29	6.53	7.08	6.95	0.13
curcumin	1	8.42	6.95	8.49	6.66	6.78	7.46	0.41
	2.5	7.43	7.05	8.37	7.14	6.56	7.31	0.30
	10	8.24	6.54	8.02	6.71	7.12	7.33	0.34
	50	7.30	6.75	7.81	6.75	6.78	7.08	0.21
	100	7.20	6.90	6.04	6.88	6.90	6.78	0.20
DMC	1	7.77	6.49	8.88	6.96	6.95	7.41	0.42
	2.5	7.34	6.41	8.58	6.62	6.56	7.10	0.40
	10	7.90	7.07	8.28	6.71	6.65	7.32	0.33
	50	8.24	6.99	7.55	7.22	7.08	7.42	0.23
	100	8.02	6.77	6.30	6.53	6.34	6.79	0.32
BDMC	1	7.34	7.08	7.29	6.58	6.99	7.06	0.14
	2.5	7.43	7.03	7.03	7.09	6.69	7.05	0.12
	10	6.74	7.12	8.24	6.84	6.90	7.17	0.28
	50	8.03	7.03	7.29	6.88	7.12	7.27	0.20
	100	8.19	6.86	7.81	6.92	6.73	7.30	0.29
curcuminoids	1	7.46	6.69	7.63	9.96	7.08	7.76	0.57
	2.5	6.94	6.60	8.24	6.88	6.99	7.13	0.29
	10	8.06	6.82	8.45	6.79	6.86	7.40	0.36
	50	8.10	7.08	6.90	6.66	6.95	7.14	0.25
	100	7.50	6.65	8.84	6.71	6.56	7.25	0.43
THC	1	7.68	6.86	6.68	6.31	7.84	7.07	0.29
	2.5	7.60	6.56	7.55	6.23	7.15	7.02	0.27
	10	6.91	6.86	7.33	6.10	7.63	6.97	0.26
	50	6.78	7.03	7.07	6.49	7.28	6.93	0.14
	100	8.92	6.95	6.73	6.14	7.46	7.24	0.47
α-tocopherol	10	7.93	6.67	7.24	6.18	7.50	7.10	0.31
	25	7.46	6.97	7.33	6.79	7.37	7.18	0.13
	100	7.76	7.01	7.55	5.84	7.28	7.09	0.34
	250	9.27	6.88	6.64	5.93	7.15	7.17	0.56
	500	8.75	6.37	6.81	6.18	7.41	7.10	0.46

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APPENDIX B

The study of the time dependent effects of curcuminoids, its derivatives and α-tocopherol on he-oxLDL.

1. The effect of 10 μM curcuminoids and its derivatives on TBARs formation.

incubation time (hr)	condition	MDA (nmol/mg protein)					Mean	S.E.M
		N1	N2	N3	N4	N5		
0	nLDL	2.10	1.93	1.99	1.79	2.22	2.00	0.07
	he-oxLDL	2.20	1.61	1.95	1.85	2.01	1.93	0.10
	curcumin	2.17	1.79	1.64	2.17	2.24	2.00	0.12
	DMC	2.12	1.75	1.88	1.95	1.94	1.93	0.06
	BDMC	2.16	1.91	1.85	1.74	2.14	1.96	0.08
	curcuminoids	2.21	1.92	1.90	1.67	2.35	2.01	0.12
	THC	2.20	1.87	1.94	2.00	2.24	2.05	0.07
	α-tocopherol	2.11	1.80	1.91	2.08	2.33	2.04	0.09
1	nLDL	2.03	2.01	1.73	2.27	2.17	2.04	0.09
	he-oxLDL	2.77	2.51	2.54	2.83	2.45	2.62	0.08
	curcumin	2.12	2.05	1.76	2.29	2.19	2.08	0.09
	DMC	2.17	2.14	1.90	2.37	2.20	2.16	0.08
	BDMC	2.29	2.21	1.96	2.36	2.25	2.22	0.07
	curcuminoids	2.13	2.02	1.80	2.28	2.21	2.09	0.08
	THC	2.10	2.03	1.74	2.29	2.17	2.07	0.09
	α-tocopherol	2.16	2.07	1.85	2.33	2.19	2.12	0.08
3	nLDL	2.22	1.97	1.89	2.14	2.23	2.09	0.07
	he-oxLDL	3.89	3.69	3.74	3.68	3.31	3.66	0.10
	curcumin	2.54	2.41	2.09	2.22	2.36	2.33	0.08
	DMC	2.66	2.50	2.44	2.58	2.53	2.54	0.04
	BDMC	2.91	2.83	2.65	2.76	2.87	2.80	0.05
	curcuminoids	2.64	2.35	2.24	2.47	2.47	2.43	0.07
	THC	2.36	2.31	2.22	2.15	2.35	2.28	0.04
	α-tocopherol	2.98	2.70	2.48	2.57	2.76	2.70	0.09
6	nLDL	2.44	2.36	1.92	2.64	2.25	2.33	0.12
	he-oxLDL	12.71	9.90	10.26	10.93	11.08	10.97	0.48
	curcumin	5.89	5.05	4.56	5.98	5.22	5.34	0.27
	DMC	6.48	5.98	6.30	6.81	7.04	6.52	0.19
	BDMC	7.09	6.26	6.61	7.57	7.09	6.92	0.22
	curcuminoids	5.71	5.22	5.79	6.13	5.36	5.64	0.16
	THC	4.70	4.66	4.91	5.61	5.48	5.07	0.20
	α-tocopherol	7.93	6.24	5.57	7.29	7.94	6.99	0.47
9	nLDL	2.96	2.54	1.92	2.80	2.43	2.53	0.18
	he-oxLDL	18.40	17.12	21.06	22.77	18.62	19.59	1.02
	curcumin	8.56	10.19	7.49	9.37	8.01	8.72	0.48
	DMC	9.88	9.91	9.67	10.32	9.17	9.79	0.19
	BDMC	12.01	10.38	9.51	11.33	10.04	10.65	0.45
	curcuminoids	8.82	8.76	7.61	8.82	7.77	8.36	0.27
	THC	7.85	7.46	6.15	7.28	6.76	7.10	0.30
	α-tocopherol	13.67	12.85	13.00	16.13	12.09	13.55	0.69
24	nLDL	3.10	2.66	2.27	3.16	2.76	2.79	0.16
	he-oxLDL	19.98	19.14	19.78	22.88	19.38	20.23	0.68
	curcumin	8.34	9.30	8.76	9.77	8.50	8.93	0.26
	DMC	10.89	11.04	12.39	9.98	9.44	10.75	0.51
	BDMC	11.92	11.36	10.90	12.41	11.97	11.71	0.26
	curcuminoids	9.77	9.59	8.70	9.05	8.22	9.07	0.28
	THC	7.98	8.35	6.81	8.32	7.15	7.72	0.31
	α-tocopherol	15.54	13.30	11.92	15.45	11.49	13.54	0.85

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2. The effect of 10 μM curcuminoids, its derivatives and α-tocopherol on α-tocopherol levels.

incubation time (hr)	condition	α-tocopherol (nmol/mg protein)					Mean	S.E.M
		N1	N2	N3	N4	N5		
0	nLDL	14.71	18.59	17.94	19.74	16.35	17.47	0.88
	he-oxLDL	14.65	18.26	17.76	20.00	16.94	17.52	0.88
	curcumin	16.00	16.45	18.58	20.27	16.11	17.48	0.84
	DMC	14.84	17.58	17.94	18.94	16.54	17.17	0.70
	BDMC	11.35	18.09	17.54	19.73	16.43	16.63	1.42
	curcuminoids	12.80	17.54	18.39	19.35	17.30	17.08	1.13
	THC	11.47	16.53	17.18	19.12	16.19	16.10	1.26
	α-tocopherol	45.73	47.37	49.39	44.43	41.03	45.59	1.41
0.5	nLDL	ND	18.87	18.23	19.28	16.22	18.15	0.68
	he-oxLDL	ND	16.5	17.27	19	15.6	17.09	0.72
	curcumin	ND	18.19	17.97	19.28	15.88	17.83	0.71
	DMC	ND	18.14	17.5	18.31	15.28	17.31	0.70
	BDMC	ND	17.55	17.75	19.11	16.05	17.62	0.63
	curcuminoids	ND	16.45	17.26	17.87	16.19	16.94	0.38
	THC	ND	18.36	17.63	19.41	15.33	17.68	0.87
	α-tocopherol	ND	44.22	39.64	36.89	33.73	38.62	2.22
1	nLDL	14.66	18.04	17.51	18.73	16.11	17.01	0.73
	he-oxLDL	10.49	15.04	15.36	17.46	13.86	14.44	1.15
	curcumin	10.12	16.55	16.65	18.33	14.99	15.33	1.41
	DMC	9.69	16.45	16.15	17.84	14.69	14.96	1.41
	BDMC	11.10	15.65	15.97	17.67	14.07	14.89	1.11
	curcuminoids	11.25	16.42	16.43	18.16	14.69	15.39	1.17
	THC	8.13	17.03	16.62	18.57	15.33	15.14	1.83
	α-tocopherol	37.76	38.32	29.19	34.82	33.42	34.70	1.65
1.5	nLDL	ND	18.05	17.94	19	15.61	17.65	0.72
	he-oxLDL	ND	13.65	11.14	15.5	11.74	13.01	0.99
	curcumin	ND	16.01	15.01	17.39	14.14	15.64	0.70
	DMC	ND	14.88	14.36	16.64	13.45	14.83	0.67
	BDMC	ND	13.55	12.91	15.84	12.29	13.65	0.77
	curcuminoids	ND	15.35	15.49	16.89	14.69	15.61	0.46
	THC	ND	16.68	15.98	17.98	14.82	16.37	0.66
	α-tocopherol	ND	35.9	22.52	32.93	28.6	29.99	2.91
2	nLDL	15.18	17.36	17.08	18.97	15.81	16.88	0.66
	he-oxLDL	9.61	11.5	9.49	11.94	9.58	10.42	0.53
	curcumin	6.91	14.73	14.18	15.48	14.58	13.18	1.58
	DMC	7.49	13.69	12.55	14.03	13.28	12.21	1.21
	BDMC	5.88	12.04	11.11	12.52	11.49	10.61	1.21
	curcuminoids	11.17	14.06	14.4	15.41	15.12	14.03	0.76
	THC	4.03	16.36	15.81	17.27	15.3	13.75	2.45
	α-tocopherol	20.07	31.93	26.75	29.64	26.47	26.97	2.00
3	nLDL	13.83	17.35	17.62	18.23	15.57	16.52	0.81
	he-oxLDL	4.23	5.21	4.65	5.37	5.12	4.92	0.21
	curcumin	12.19	13.89	13.86	13.56	13.11	13.32	0.32
	DMC	16.70	13.13	11.5	11.99	11.5	12.96	0.98
	BDMC	9.75	11.67	10.42	11.12	10.14	10.62	0.34
	curcuminoids	12.66	14.23	13.21	13.9	12.81	13.36	0.31
	THC	7.64	15.13	15.07	16.43	14.93	13.84	1.57
	α-tocopherol	24.67	20.52	19.07	27.42	22.57	22.85	1.48

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incubation time (hr)	condition	α-tocopherol (nmol/mg protein)					Mean	S.E.M
		N1	N2	N3	N4	N5		
4	nLDL	13.68	17.58	17.15	18.34	15.59	16.47	0.83
	he-oxLDL	3.21	5.49	0	0	4.85	2.71	1.17
	curcumin	8.18	13.7	12.57	13.04	10.15	11.53	1.03
	DMC	6.77	12.77	10.54	10.62	8.58	9.86	1.02
	BDMC	4.78	10.57	9.38	8.72	7.40	8.17	0.99
	curcuminoids	10.06	13.37	12.11	12.41	9.59	11.51	0.72
	THC	6.27	15.16	13.76	15.61	12.85	12.73	1.69
	α-tocopherol	21.31	19.88	18.37	25.13	16.23	20.18	1.50
5	nLDL	14.94	17.86	17.40	18.28	15.92	16.88	0.63
	he-oxLDL	3.01	0	0	0	4.90	1.58	1.01
	curcumin	9.95	13.14	10.49	10.54	8.90	10.60	0.70
	DMC	5.80	12.04	9.32	8.8	7.68	8.73	1.02
	BDMC	4.74	8.72	8.08	7.89	6.78	7.24	0.70
	curcuminoids	9.17	13.23	10.28	10.09	9.13	10.38	0.75
	THC	3.05	14.06	12.92	13.68	11.5	11.04	2.05
	α-tocopherol	15.12	13.23	14.28	18.98	15.27	15.38	0.97
6	nLDL	13.67	17.78	16.84	18.13	15.70	16.42	0.81
	he-oxLDL	3.58	0	0	0	0	0.72	0.72
	curcumin	9.75	11.08	8.24	9.11	8.02	9.24	0.55
	DMC	11.48	9.91	6.78	7.02	7	8.44	0.96
	BDMC	7.90	6.99	5.45	5.98	6.10	6.48	0.43
	curcuminoids	10.69	10.31	7.74	8.79	8.90	9.29	0.54
	THC	6.93	13.19	11.81	11.81	10.36	10.82	1.07
	α-tocopherol	15.43	13.03	11.92	15.9	13.69	13.99	0.74
7	nLDL	13.67	17.71	17.4	17.88	16.00	16.53	0.79
	he-oxLDL	0	0	0	0	0	0	0
	curcumin	4.05	9.97	7.41	7.21	7.74	7.28	0.95
	DMC	2.90	8.72	5.7	6.32	7.03	6.13	0.95
	BDMC	2.77	6.9	0	5.63	5.44	4.15	1.24
	curcuminoids	3.67	10.71	7.12	7.66	8.02	7.44	1.13
	THC	5.03	12.04	10.6	10.16	10.01	9.57	1.19
	α-tocopherol	10.37	12.7	11.43	9.91	11.09	11.10	0.48
8	nLDL	13.43	17.08	17.41	18.29	15.57	16.36	0.85
	he-oxLDL	0	0	0	0	0	0	0
	curcumin	3.67	9.91	6.79	7.24	6.39	6.80	1.00
	DMC	2.84	8.4	4.68	5.69	5.84	5.49	0.90
	BDMC	0	0	0	0	0	0	0
	curcuminoids	3.67	9.75	5.8	7.03	7.06	6.66	0.99
	THC	5.03	11.41	9.98	9.77	9.73	9.18	1.08
	α-tocopherol	8.01	10.35	9.66	9.2	10.2	9.48	0.42
9	nLDL	14.01	16.35	16.76	17.88	15.25	16.05	0.66
	he-oxLDL	0	0	0	0	0	0	0
	curcumin	3.30	8.43	5.4	5.98	6.08	5.84	0.82
	DMC	0	0	0	0	0	0	0
	BDMC	0	0	0	0	0	0	0
	curcuminoids	0	7.4	5.81	6.18	6.48	5.17	1.32
	THC	3.20	9.36	9.64	8.86	9.46	8.10	1.23
	α-tocopherol	6.99	8.74	6.72	8.17	7.4	7.60	0.37

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incubation time (hr)	condition	α-tocopherol (nmol/mg protein)					Mean	S.E.M
		N1	N2	N3	N4	N5		
24	nLDL	12.91	16.46	16.44	18.2	14.95	15.79	0.79
	he-oxLDL	0	0	0	0	0	0	0
	curcumin	2.76	6.6	4.67	5.9	5.5	5.09	0.59
	DMC	0	0	0	0	0	0	0
	BDMC	0	0	0	0	0	0	0
	curcuminoids	0	0	5.43	5.85	5.89	3.43	1.26
	THC	2.82	8.28	8.27	8.07	8.54	7.20	0.98
	α-tocopherol	0	6.58	5.4	0	5.49	3.49	1.29

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3. Effect of 10 μM curcuminoids, its derivatives and α-tocopherol on rate of TBARs formation in he-oxLDL during 0-3 hr of incubation time

condition	rate of TBARs formation (nmol/mg protein hr^{-1})					Mean	S.E.M
	N1	N2	N3	N4	N5		
nLDL	0.041	0.014	-0.031	0.117	0.002	0.029	0.025
he-oxLDL	0.566	0.694	0.596	0.608	0.431	0.579	0.043
curcumin	0.125	0.206	0.150	0.017	0.040	0.108	0.035
DMC	0.180	0.252	0.187	0.211	0.195	0.205	0.013
BDMC	0.249	0.307	0.268	0.339	0.244	0.281	0.018
curcuminoids	0.141	0.143	0.113	0.268	0.041	0.141	0.037
THC	0.051	0.145	0.092	0.051	0.035	0.075	0.020
α-tocopherol	0.291	0.299	0.189	0.165	0.144	0.218	0.033

4. Effect of 10 μM curcuminoids, its derivatives and α-tocopherol on rate of TBARs formation in he-oxLDL during 3-9 hr of incubation time.

condition	rate of TBARs formation (nmol/mg protein hr^{-1})					Mean	S.E.M
	N1	N2	N3	N4	N5		
nLDL	0.123	0.095	0.005	0.110	0.035	0.074	0.023
he-oxLDL	2.418	2.239	2.887	3.182	2.553	2.655	0.169
curcumin	1.003	0.974	0.899	1.191	0.942	1.002	0.050
DMC	1.203	1.234	1.205	1.291	1.107	1.208	0.030
BDMC	1.517	1.259	1.142	1.429	1.195	1.308	0.071
curcuminoids	1.031	1.069	0.896	1.057	0.884	0.987	0.040
THC	0.581	0.858	0.655	0.855	0.736	0.504	0.047
α-tocopherol	1.781	1.692	1.753	2.261	1.555	1.808	0.120

5. Effect of 10 μM curcuminoids, its derivatives and α-tocopherol on rate of TBARs formation in he-oxLDL during 9-24 hr of incubation time.

condition	rate of TBARs formation (nmol/mg protein hr^{-1})					Mean	S.E.M
	N1	N2	N3	N4	N5		
nLDL	0.010	0.007	0.023	0.024	0.022	0.017	0.004
he-oxLDL	0.106	0.135	-0.085	0.008	0.050	0.043	0.039
curcumin	-0.014	0.069	0.085	0.026	0.033	0.040	0.017
DMC	0.067	0.076	0.181	-0.023	0.018	0.064	0.034
BDMC	-0.006	0.065	0.093	0.072	0.129	0.071	0.022
curcuminoids	0.063	0.055	0.073	0.015	0.030	0.047	0.011
THC	0.008	0.060	0.044	0.069	0.026	0.041	0.011
α-tocopherol	0.125	0.029	-0.072	-0.046	-0.040	-0.001	0.006

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6. Effect of 10 μM curcuminoids and its derivatives on decreasing rate of α-tocopherol levels in he-oxLDL during 0-3 hr of incubation time.

condition	decreasing rate of α-tocopherol ($x 10^{-2}$ nmol/mg protein min^{-1})					Mean	S.E.M
	N1	N2	N3	N4	N5		
nLDL	0.494	0.690	0.175	0.842	0.429	0.526	0.114
he-oxLDL	5.788	7.251	7.282	5.797	6.570	6.538	0.330
curcumin	5.122	1.423	2.623	3.730	2.797	3.139	0.617
DMC	4.975	2.468	3.574	3.862	2.797	3.535	0.440
BDMC	5.210	3.567	3.957	4.783	3.498	4.203	0.340
curcuminoids	3.835	1.840	2.879	3.029	2.500	2.817	0.327
THC	2.691	0.775	1.173	1.492	0.697	1.366	0.361

7. Effect of 10 μM curcuminoids and its derivatives on decreasing rate of α-tocopherol levels in he-oxLDL during 3 to time that α-tocopherol levels decreased until undetectable.

condition	decreasing rate of α-tocopherol ($x 10^{-2}$ nmol/mg protein min^{-1})					Mean	S.E.M
	N1	N2	N3	N4	N5		
nLDL	0.073	0.071	0.094	0.002	0.050	0.058	0.016
he-oxLDL	1.763	4.338	0.000	7.968	2.842	3.382	1.347
curcumin	0.418	0.579	0.730	0.607	0.604	0.588	0.050
DMC	1.634	3.648	2.276	2.103	3.196	2.571	0.370
BDMC	1.786	3.891	4.341	2.288	3.380	3.137	0.481
curcuminoids	2.576	1.129	0.618	0.638	0.549	1.102	0.383
THC	0.570	0.543	0.540	0.663	0.507	0.565	0.027

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8. Effect of 10 μM curcuminoids, its derivatives and α-tocopherol on % damage of CA in he-oxLDL.

incubation time (hr)	condition	% damage of CA					Mean	S.E.M
		N1	N2	N3	N4	N5		
1	nLDL	0.00	0.01	0.00	0.01	-0.01	0.00	0.00
	he-oxLDL	1.71	1.53	4.59	-1.66	-4.30	0.37	1.53
	curcumin	-0.05	1.85	-3.91	0.26	0.94	-0.18	0.99
	DMC	0.49	-1.21	3.22	0.18	-0.69	0.40	0.77
	BDMC	0.61	0.45	9.59	1.62	1.09	2.67	1.74
	curcuminoids	0.34	-0.23	-0.48	2.16	-6.09	-0.86	1.39
	THC	-0.03	2.44	-5.10	0.89	-1.21	-0.60	1.27
	α-tocopherol	0.59	-0.40	0.94	-0.62	-3.11	-0.52	0.71
3	nLDL	-0.02	-0.01	0.00	0.00	0.01	0	0
	he-oxLDL	6.37	9.53	11.08	8.04	13.18	9.64	1.18
	curcumin	1.34	2.82	2.57	1.14	1.36	1.84	0.35
	DMC	2.47	4.59	4.61	2.36	4.02	3.61	0.50
	BDMC	3.95	6.09	4.96	4.45	6.12	5.11	0.43
	curcuminoids	0.66	1.42	2.06	2.56	3.54	2.04	0.49
	THC	0.11	0.76	1.46	1.20	1.12	0.92	0.23
	α-tocopherol	4.53	6.14	6.37	4.54	7.01	5.71	0.50
6	nLDL	0.00	-0.02	-0.01	0.01	-0.01	-0.01	0.01
	he-oxLDL	19.74	12.86	17.91	14.92	37.19	20.52	4.33
	curcumin	6.95	3.71	7.34	4.51	3.79	5.26	0.78
	DMC	12.55	6.46	9.28	9.13	11.30	9.75	1.04
	BDMC	13.30	9.04	12.42	10.67	17.24	12.53	1.39
	curcuminoids	3.38	2.53	7.57	5.77	9.96	5.84	1.36
	THC	2.40	2.14	4.86	3.19	3.12	3.14	0.47
	α-tocopherol	13.49	9.57	10.45	11.30	23.46	13.65	2.54
9	nLDL	-0.01	0.01	0.00	0.01	0.00	0.00	0.00
	he-oxLDL	24.28	30.07	27.81	25.54	34.02	28.34	1.73
	curcumin	12.11	12.37	11.40	10.21	7.65	10.75	0.86
	DMC	16.43	19.55	14.42	12.91	15.42	15.75	1.11
	BDMC	17.41	23.11	19.29	16.43	18.53	18.95	1.15
	curcuminoids	8.26	8.74	11.78	8.19	9.14	9.22	0.66
	THC	6.19	6.68	7.72	6.93	12.31	7.97	1.12
	α-tocopherol	19.52	25.43	19.57	18.92	22.26	21.14	1.22
24	nLDL	0.00	-0.01	0.01	-0.01	0.01	0.00	0.00
	he-oxLDL	27.34	32.14	29.50	22.35	33.06	28.88	1.92
	curcumin	14.55	14.91	10.18	8.12	13.93	12.34	1.35
	DMC	21.69	21.42	19.04	13.31	20.43	19.18	1.54
	BDMC	22.77	26.54	20.10	17.09	21.40	21.58	1.56
	curcuminoids	10.06	10.49	10.80	6.29	12.94	10.12	1.08
	THC	7.79	7.49	6.62	5.87	11.87	7.93	1.04
	α-tocopherol	22.95	22.98	20.31	17.98	26.94	22.23	1.50

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9. Effect of 10 μM curcuminoids, its derivatives and α-tocopherol on % damage of CL in he-oxLDL.

incubation time (hr)	condition	% damage of CL					mean	S.E.M
		N1	N2	N3	N4	N5		
1	nLDL	0	0	0	0	0	0	0
	he-oxLDL	4.88	-1.39	3.06	1.57	-3.39	0.95	1.49
	curcumin	0.57	-0.03	0.05	-0.51	0.80	0.18	0.23
	DMC	1.25	1.44	1.38	2.97	-0.79	1.25	0.60
	BDMC	1.50	1.22	4.87	0.03	-3.14	0.90	1.29
	curcuminoids	0.14	0.21	0.42	2.57	-4.74	-0.28	1.20
	THC	0.09	5.29	4.78	0.00	-3.77	1.28	1.69
	α-tocopherol	1.29	5.29	3.88	1.06	-5.50	1.20	1.86
3	nLDL	0	0	0	0	0	0	0
	he-oxLDL	6.00	10.06	9.97	5.69	11.65	8.67	1.20
	curcumin	0.91	1.17	2.58	1.42	3.91	2.00	0.56
	DMC	1.93	2.58	3.82	2.49	5.73	3.31	0.68
	BDMC	3.59	3.09	4.82	3.25	8.09	4.57	0.93
	curcuminoids	0.19	0.29	2.80	1.84	3.51	1.73	0.66
	THC	-0.06	0.19	1.62	0.68	2.86	1.06	0.53
	α-tocopherol	2.59	5.67	8.08	3.24	9.02	5.72	1.27
6	nLDL	0	0	0	0	0	0	0
	he-oxLDL	11.11	26.67	16.42	19.28	17.91	18.28	2.51
	curcumin	2.42	5.43	4.30	5.50	5.89	4.71	0.63
	DMC	5.32	10.52	6.84	7.35	7.87	7.58	0.85
	BDMC	6.74	16.68	8.71	14.07	9.60	11.16	1.83
	curcuminoids	2.42	6.61	5.77	2.86	5.38	4.61	0.83
	THC	1.08	0.81	4.55	2.44	3.98	2.57	0.75
	α-tocopherol	7.10	17.29	11.73	13.06	13.49	12.54	1.64
9	nLDL	0	0	0	0	0	0	0
	he-oxLDL	19.42	24.32	25.68	18.69	21.44	21.91	1.36
	curcumin	8.73	8.76	9.55	5.83	7.23	8.02	0.66
	DMC	10.79	11.47	13.47	9.00	13.60	11.67	0.86
	BDMC	12.02	15.23	16.73	13.69	15.38	14.61	0.81
	curcuminoids	7.76	10.38	8.77	6.16	7.65	8.14	0.70
	THC	2.77	5.13	7.55	3.51	8.42	5.48	1.10
	α-tocopherol	14.44	15.23	14.96	12.40	14.47	14.30	0.50
24	nLDL	0	0	0	0	0	0	0
	he-oxLDL	21.87	25.07	24.86	22.26	27.14	24.24	0.97
	curcumin	11.58	10.26	9.68	8.01	8.91	9.69	0.60
	DMC	13.40	14.64	12.70	11.34	15.25	13.46	0.69
	BDMC	16.39	18.48	18.54	14.29	16.83	16.91	0.78
	curcuminoids	8.84	8.05	6.08	7.63	5.94	7.31	0.57
	THC	5.04	4.58	4.41	4.48	8.45	5.39	0.77
	α-tocopherol	18.79	18.96	15.97	16.80	19.27	17.96	0.66

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10. Effect of 10 μM curcuminoids, its derivatives and α-tocopherol on CL/CO ratio in he-oxLDL.

incubation time (hr)	Tested compounds	% inhibition of dereasing CL/CO ratio					Mean	S.E.M
		N1	N2	N3	N4	N5		
1	curcumin	75.20	87.87	79.82	90.63	58.20	78.34	5.74
	DMC	54.14	47.99	65.23	71.74	43.32	56.48	5.29
	BDMC	63.08	58.52	42.19	88.34	52.35	60.90	7.70
	curcuminoids	54.40	-21.93	64.40	48.10	65.51	42.10	16.33
	THC	81.85	70.15	52.61	106.89	63.81	75.06	9.26
	α-tocopherol	33.28	61.05	46.18	33.62	79.53	50.73	8.82
3	curcumin	71.19	66.90	52.28	87.37	63.46	68.24	5.72
	DMC	42.71	57.93	50.32	44.88	43.18	47.80	2.87
	BDMC	43.25	33.75	53.16	26.38	32.74	37.86	4.68
	curcuminoids	53.79	76.00	62.68	55.15	65.08	62.54	3.99
	THC	79.73	88.57	82.19	63.12	66.08	75.94	4.87
	α-tocopherol	22.51	29.55	44.92	35.07	40.40	34.49	3.95
6	curcumin	60.62	77.75	87.95	83.32	66.50	75.23	5.12
	DMC	39.82	54.54	49.67	62.42	40.76	49.44	4.26
	BDMC	24.84	38.80	53.84	21.16	35.43	34.81	5.76
	curcuminoids	48.00	70.19	76.35	91.92	63.17	69.93	7.25
	THC	71.20	85.54	82.28	100.44	91.03	86.10	4.83
	α-tocopherol	15.19	51.52	44.13	42.21	39.49	38.51	6.16
9	curcumin	54.05	68.03	84.46	73.38	66.17	69.22	4.95
	DMC	41.35	60.19	71.40	57.61	49.89	56.09	5.05
	BDMC	37.08	53.05	53.27	56.49	33.96	46.77	4.66
	curcuminoids	54.57	55.90	86.86	79.47	62.39	67.84	6.50
	THC	62.86	84.82	90.58	82.74	65.64	77.33	5.51
	α-tocopherol	26.69	46.18	69.41	38.75	54.99	47.20	7.23
24	curcumin	65.75	53.38	63.22	69.55	67.41	63.86	2.82
	DMC	41.82	30.57	44.54	34.35	28.22	35.90	3.16
	BDMC	38.91	22.53	17.59	49.34	10.99	27.87	7.08
	curcuminoids	60.35	75.48	60.47	69.53	93.38	71.84	6.10
	THC	68.20	88.64	69.25	85.80	75.89	77.55	4.18
	α-tocopherol	23.28	33.99	24.62	42.32	38.36	32.51	3.74

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11. Effect of 10 μM curcumin, its derivatives and α-tocopherol on oxidized lipid products formation in he-oxLDL at RT 9.6 min.

incubation time (hr)	condition	AUC of oxidized lipid product at RT 9.6 min					Mean	S.E.M
		N1	N2	N3	N4	N5		
0	nLDL	0	0	0	0	0	0	0
	he-oxLDL	1102	1145	2116	0	0	873	400
	curcumin	0	0	0	0	0	0	0
	DMC	1287	0	1806	2100	0	1039	444
	BDMC	0	0	0	0	1452	290	290
	curcuminoids	0	0	1477	0	0	295	295
	THC	1754	0	0	0	0	351	351
	α-tocopherol	0	0	0	1357	0	271	271
1	nLDL	0	0	1895	0	0	379	379
	he-oxLDL	4721	0	4985	2981	4721	3482	941
	curcumin	0	0	1587	1557	0	629	385
	DMC	0	0	2052	1987	0	808	495
	BDMC	1423	0	2215	1547	0	1037	444
	curcuminoids	0	0	1965	0	0	393	393
	THC	0	0	1584	0	0	317	317
	α-tocopherol	1145	0	2756	1356	0	1051	511
3	nLDL	1113	0	2541	0	1113	953	468
	he-oxLDL	4646	0	7214	5614	4646	4424	1202
	curcumin	1542	0	3126	1565	1326	1512	496
	DMC	2563	0	4012	1847	1495	1983	657
	BDMC	1958	0	4195	2385	1598	2027	676
	curcuminoids	1432	0	2911	1142	0	1097	539
	THC	1242	0	2475	1356	0	1015	467
	α-tocopherol	2054	0	5001	1954	1321	2066	820
6	nLDL	4018	0	0	3145	4018	2236	927
	he-oxLDL	18587	25367	12858	23653	18587	19810	2202
	curcumin	4498	3610	3511	4654	4498	4154	245
	DMC	6551	5874	4620	5698	6551	5859	355
	BDMC	5858	8746	6511	8561	5858	7107	643
	curcuminoids	5344	9373	5107	4568	4344	5747	924
	THC	5267	4240	9640	3654	7267	6014	1095
	α-tocopherol	9435	10160	6832	9025	9435	8977	567
9	nLDL	3426	0	3806	2614	3426	2654	692
	he-oxLDL	36877	39653	46830	39517	26877	37951	3225
	curcumin	14483	8263	9178	8652	6483	9412	1346
	DMC	19201	14475	14214	15253	7201	14069	1938
	BDMC	23906	22695	16547	18521	9906	18315	2493
	curcuminoids	18249	13247	9877	6584	7249	11041	2150
	THC	16149	10014	8898	5698	7149	9582	1800
	α-tocopherol	29641	25632	26369	19321	8641	21921	3717
24	nLDL	4803	1965	0	5234	4803	3361	1023
	he-oxLDL	42536	43516	55092	50659	32536	44868	3856
	curcumin	14652	10632	9183	21659	14853	14196	2170
	DMC	19745	14652	9680	25639	17852	17514	2652
	BDMC	31154	23677	14137	29871	20523	23872	3123
	curcuminoids	21854	10054	8631	24542	10852	15187	3317
	THC	18654	8965	5632	20651	12874	13355	2833
	α-tocopherol	28032	24145	20013	19523	22032	22749	1554

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RT 10.3 min

incubation time (hr)	condition	AUC of oxidized lipid product at RT 10.3 min					Mean	S.E.M
		N1	N2	N3	N4	N5		
0	nLDL	1194	0	1194	1194	0	716	292
	he-oxLDL	0	0	1467	0	0	293	293
	curcumin	0	0	0	0	0	0	0
	DMC	0	0	1196	0	0	239	239
	BDMC	0	0	0	0	0	0	0
	curcuminoids	0	0	0	0	0	0	0
	THC	0	0	1451	0	0	290	290
	α-tocopherol	0	0	1375	0	1008	477	298
1	nLDL	0	0	0	0	0	0	0
	he-oxLDL	2042	0	1895	1987	2042	1593	399
	curcumin	0	0	0	0	0	0	0
	DMC	0	0	0	0	0	0	0
	BDMC	0	0	1123	0	0	225	225
	curcuminoids	0	0	0	0	0	0	0
	THC	0	0	0	0	0	0	0
	α-tocopherol	1423	0	0	1325	0	550	337
3	nLDL	0	0	0	0	0	0	0
	he-oxLDL	2958	0	3865	3715	2958	2699	700
	curcumin	0	0	1142	0	0	228	228
	DMC	0	0	2562	1298	0	772	513
	BDMC	1765	0	1785	1165	0	943	401
	curcuminoids	0	0	1195	0	0	239	239
	THC	0	0	1234	0	0	247	247
	α-tocopherol	1892	0	1562	1323	0	955	400
6	nLDL	2449	0	0	0	0	490	490
	he-oxLDL	4647	3285	3145	4021	2314	3482	398
	curcumin	2517	0	0	0	0	503	503
	DMC	2929	1074	1064	0	0	1013	535
	BDMC	3119	1253	1485	1426	1252	1707	356
	curcuminoids	2543	0	1055	0	0	720	500
	THC	2476	0	1178	0	0	731	492
	α-tocopherol	3832	1070	1847	1562	1563	1975	481
9	nLDL	2407	0	0	1524	0	786	501
	he-oxLDL	4003	2714	6281	5321	7859	5236	891
	curcumin	2577	0	1144	0	0	744	509
	DMC	3538	1423	3958	2541	0	2292	721
	BDMC	3825	0	2413	2658	0	1779	765
	curcuminoids	2481	0	0	0	0	496	496
	THC	2475	0	0	0	0	495	495
	α-tocopherol	3046	1942	3559	1547	0	2019	622
24	nLDL	2721	0	0	1147	1118	1118	521
	he-oxLDL	6726	3875	8572	6985	7177	7177	990
	curcumin	2814	0	1399	1236	1463	1463	457
	DMC	3162	1462	1200	1574	2073	2073	410
	BDMC	5425	0	2716	1985	2655	2655	878
	curcuminoids	3211	0	1293	1532	1858	1858	619
	THC	2614	0	0	1458	1220	1220	531
	α-tocopherol	4152	1765	6979	2792	3696	3696	904

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RT 11.6 min

incubation time (hr)	condition	AUC of oxidized lipid product at RT 11.6 min					Mean	S.E.M
		N1	N2	N3	N4	N5		
0	nLDL	0	0	0	0	0	0	0
	he-oxLDL	0	0	0	0	0	0	0
	curcumin	0	0	0	0	0	0	0
	DMC	0	0	0	0	0	0	0
	BDMC	0	0	0	0	0	0	0
	curcuminoids	0	0	1826	0	0	365	365
	THC	0	0	3640	0	0	728	728
	α-tocopherol	0	0	1022	0	0	204	204
1	nLDL	0	0	1149	0	0	230	
	he-oxLDL	0	0	3745	2345	0	1218	778
	curcumin	0	0	1325	1237	0	512	314
	DMC	0	0	1654	1654	0	662	405
	BDMC	0	0	1799	1785	0	717	439
	curcuminoids	0	0	1542	0	0	308	308
	THC	0	0	1479	0	0	296	296
	α-tocopherol	0	0	1865	1157	0	604	387
3	nLDL	5679	0	0	0	0	1136	1136
	he-oxLDL	7520	0	4123	2961	2520	3425	1225
	curcumin	6095	0	0	0	0	1219	1219
	DMC	6874	0	1342	0	0	1643	1333
	BDMC	6563	0	1542	1134	0	1848	1218
	curcuminoids	5623	0	1754	0	0	1475	1091
	THC	6521	0	0	0	0	1304	1304
	α-tocopherol	6742	0	1952	1275	0	1994	1245
6	nLDL	2871	0	0	0	1007	776	559
	he-oxLDL	6007	4352	4435	3561	4871	4645	401
	curcumin	3664	0	1931	0	1964	1512	692
	DMC	4075	1574	2543	0	1568	1952	669
	BDMC	4285	1857	3011	0	1541	2139	720
	curcuminoids	4106	1131	1954	0	1606	1759	673
	THC	2906	0	1409	0	1606	1184	548
	α-tocopherol	3942	1253	1167	1325	2542	2046	537
9	nLDL	0	0	0	0	1154	231	231
	he-oxLDL	5577	6486	6486	5026	5226	5700	278
	curcumin	0	0	0	1235	1236	494	303
	DMC	3045	0	0	1865	1324	1247	580
	BDMC	3542	1112	1112	2143	1765	1712	584
	curcuminoids	0	0	0	1345	1987	666	421
	THC	0	0	0	0	1365	273	273
	α-tocopherol	4709	0	0	2503	2994	2332	785
24	nLDL	0	0	0	1325	0	265	265
	he-oxLDL	7778	5871	6865	6514	6778	6761	308
	curcumin	0	0	0	1499	0	300	300
	DMC	0	0	0	1856	1132	598	383
	BDMC	2354	0	0	2578	1452	1277	554
	curcuminoids	0	0	0	2365	0	473	473
	THC	0	0	0	1598	0	320	320
	α-tocopherol	5237	0	1502	3014	2531	2457	866

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RT 12.3 min

incubation time (hr)	condition	AUC of oxidized lipid product at RT 12.3 min					Mean	S.E.M
		N1	N2	N3	N4	N5		
0	nLDL	0	2307	0	0	1325	726	471
	he-oxLDL	0	0	2572	0	0	514	514
	curcumin	0	0	0	1140	0	228	228
	DMC	0	0	0	0	0	0	0
	BDMC	1235	3926	0	0	0	1032	762
	curcuminoids	0	0	2211	0	1247	692	450
	THC	0	0	0	1175	0	235	235
	α-tocopherol	1178	0	1843	0	0	604	385
1	nLDL	0	0	1254	0	0	251	
	he-oxLDL	1162	0	3251	1954	1162	1506	536
	curcumin	0	0	1564	0	0	313	313
	DMC	0	0	2013	0	0	403	403
	BDMC	0	0	1575	0	0	315	315
	curcuminoids	0	0	1554	0	0	311	311
	THC	0	0	1212	0	0	242	242
	α-tocopherol	0	0	1954	0	0	391	391
3	nLDL	1692	0	1546	0	0	648	397
	he-oxLDL	3086	0	5614	4198	3086	3197	924
	curcumin	2024	0	1452	0	0	695	435
	DMC	2721	0	2554	0	0	1055	647
	BDMC	2574	0	1799	1212	0	1117	505
	curcuminoids	1774	0	2014	1454	0	1048	437
	THC	1885	0	1567	1235	0	937	396
	α-tocopherol	2687	0	2121	1654	0	1292	552
6	nLDL	0	2985	0	0	0	597	597
	he-oxLDL	8855	13632	6688	8855	10395	9685	1150
	curcumin	1009	3287	1314	3909	2365	2377	556
	DMC	2169	4521	2651	1869	4251	3092	544
	BDMC	3374	6867	2877	1874	4541	3907	856
	curcuminoids	1127	3584	1163	1827	2361	2012	455
	THC	1659	3885	1773	2659	2633	2522	400
	α-tocopherol	4139	7554	2952	4139	6854	5128	882
9	nLDL	1369	0	0	1379	1369	823	336
	he-oxLDL	25096	18925	14996	26743	15096	20171	2465
	curcumin	6693	5253	3402	9652	6693	6339	1026
	DMC	9780	6965	7245	13214	9780	9397	1127
	BDMC	8917	8457	10285	16541	10917	11023	1450
	curcuminoids	8540	5874	4231	5254	4540	5688	768
	THC	7950	4587	0	3658	6950	4629	1393
	α-tocopherol	20015	13521	3391	14125	9015	12013	2776
24	nLDL	4006	2595	0	2458	4006	2613	733
	he-oxLDL	30010	23429	25067	32623	20010	26228	2270
	curcumin	9412	9562	1558	10698	5621	7370	1687
	DMC	13754	13149	4987	15521	10541	11590	1834
	BDMC	14531	14854	9158	18529	14133	14241	1495
	curcuminoids	13654	6985	4285	9254	9285	8693	1543
	THC	7165	5870	2263	12395	7547	7048	1630
	α-tocopherol	25305	15948	10682	16587	12305	16165	2537

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12. Effect of curcumin, its derivatives and α-tocopherol 10 μM on total cholesterol in he-oxLDL.

incubation time (hr)	condition	Total cholesterol (μmol/mg protein)					Mean	S.E.M
		N1	N2	N3	N4	N5		
0	nLDL	3.79	3.88	4.41	2.81	3.21	3.62	0.28
	he-oxLDL	3.97	4.06	4.37	2.96	3.00	3.67	0.29
	curcumin	3.75	4.10	4.20	2.83	3.19	3.61	0.26
	DMC	3.58	3.97	4.50	2.64	3.17	3.57	0.32
	BDMC	3.88	4.27	4.37	2.36	3.08	3.59	0.38
	curcuminoids	3.36	4.36	3.98	2.62	3.00	3.46	0.31
	THC	3.62	4.23	4.50	3.00	3.30	3.73	0.28
	α-tocopherol	3.88	4.18	4.54	2.72	3.26	3.72	0.32
1	nLDL	4.22	4.14	4.41	3.18	3.41	3.87	0.24
	he-oxLDL	3.92	4.06	4.54	2.94	3.36	3.76	0.28
	curcumin	3.84	3.84	4.46	3.00	3.21	3.67	0.25
	DMC	3.75	4.27	3.98	2.92	3.08	3.60	0.26
	BDMC	3.97	4.36	4.33	2.96	3.08	3.74	0.30
	curcuminoids	3.66	4.01	4.41	2.92	3.13	3.63	0.27
	THC	3.84	3.97	4.54	2.98	2.93	3.65	0.30
	α-tocopherol	3.62	4.23	4.54	3.18	3.06	3.73	0.29
3	nLDL	4.05	4.14	4.24	2.70	3.15	3.66	0.30
	he-oxLDL	4.09	4.10	4.11	2.81	3.17	3.66	0.27
	curcumin	3.92	4.06	4.20	2.83	3.21	3.64	0.26
	DMC	3.71	4.44	4.28	3.05	3.13	3.72	0.28
	BDMC	3.88	4.27	4.41	2.94	3.15	3.73	0.29
	curcuminoids	3.49	4.10	4.46	3.05	3.00	3.62	0.28
	THC	3.58	4.06	4.20	2.96	2.31	3.42	0.35
	α-tocopherol	3.66	3.93	3.98	3.00	2.98	3.51	0.22
6	nLDL	3.36	4.27	4.41	2.68	3.15	3.58	0.33
	he-oxLDL	3.53	4.01	4.33	2.66	3.00	3.51	0.31
	curcumin	3.84	4.10	4.50	2.51	3.13	3.61	0.35
	DMC	3.40	3.93	4.54	2.62	3.19	3.54	0.32
	BDMC	3.49	4.23	4.20	2.79	3.17	3.58	0.28
	curcuminoids	3.75	3.80	3.81	3.00	3.13	3.50	0.18
	THC	3.66	4.18	4.41	2.70	3.39	3.67	0.30
	α-tocopherol	4.01	4.10	4.33	2.90	3.28	3.72	0.27
9	nLDL	3.71	3.93	4.24	3.00	3.28	3.63	0.20
	he-oxLDL	3.58	4.06	4.15	2.96	3.21	3.59	0.20
	curcumin	3.97	3.97	4.37	2.85	3.08	3.65	0.26
	DMC	3.62	4.23	4.54	2.94	3.17	3.70	0.27
	BDMC	4.01	4.10	4.46	2.62	3.41	3.72	0.29
	curcuminoids	3.79	4.01	3.81	2.87	3.39	3.58	0.18
	THC	3.66	3.88	3.72	3.00	3.36	3.53	0.14
	α-tocopherol	3.36	4.31	4.37	2.92	3.28	3.65	0.26
24	nLDL	3.36	4.23	4.28	2.83	3.30	3.60	0.25
	he-oxLDL	4.01	4.10	4.03	2.64	3.21	3.60	0.26
	curcumin	3.19	4.18	4.11	2.94	3.41	3.57	0.22
	DMC	3.79	4.31	4.37	2.75	3.13	3.67	0.28
	BDMC	3.66	4.06	4.28	2.96	3.30	3.65	0.21
	curcuminoids	3.58	3.93	4.20	2.62	3.21	3.51	0.25
	THC	3.45	4.40	3.68	2.72	3.06	3.46	0.25
	α-tocopherol	3.53	4.18	3.81	2.85	3.30	3.54	0.20

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