# CHAPTER III: RESULTS

## 3.1 General parameters comparison between healthy with normolipidemic and diabetic with hyperlipidemic groups

All samples were analyzed on Hitachi 910 autoanalyzer with Boehringer Mannheim original reagents. LDL-cholesterol was calculated using Friedewald's formula: [LDL-cholesterol]=[total cholesterol]-[HDL cholesterol]-([triglycerides]/5).

Age, sex ratio, fasting blood glucose and lipid profile levels as mean ±SD in ten healthy with normolipidemic and diabetes with hyperlipidemic group were shown in Table 9. There were no significant difference in age and sex ratio between both group (44.7±1.25 *vs.* 45.9±2.18, 5/5 *vs.* 5/5, respectively, $P>0.05$). The levels of serum total cholesterol, triglyceride and LDL-cholesterol in diabetic group were significantly higher than those in healthy group (287.0±14.45 *vs.* 166.8±13.32, 190.2±9.93 *vs* 97.5±14.15, and 202±32.6 *vs.* 99±29.0, respectively, $P<0.05$), but HDL-cholesterol levels were significantly lower in diabetic group than those in healthy group (41.5±3.17 *vs.* 48.1±5.25, $P<0.05$). The levels of fasting blood glucose in diabetic group were significantly higher than those in healthy group (168.2±17.61 *vs.* 97.1±8.22, $P<0.05$).

**Table 8.** General parameters comparison between healthy with normolipidemic and diabetes with hyperlipidemic groups

| Parameters | Healthy with normolipidemic group (mean±SD, n=10) | Diabetic with hyperlipidemic group (mean±SD, n=10) |
|---|---|---|
| Age (yr) | 44.7±1.25 | 45.9±2.18 |
| Sex (M/F) | 5/5 | 5/5 |
| Glucose (mg/dl) | 97.1±8.22 | 168.2±17.61* |
| Cholesterol (mg/dl) | 166.8±13.32 | 287.0±14.45* |
| Triglyceride (mg/dl) | 97.5±14.15 | 190.2±9.93* |
| HDL-C (mg/dl) | 48.1±5.25 | 41.5±3.17* |
| LDL-C (mg/dl) | 99±29.0 | 202±32.60* |

*$P < 0.05$ by Student's $t$ test

**3.2 Total antioxidant capacity (TAC) of curcuminoids, α- tocopherol, and ascorbic acid**

Lag time of oxidation of Trolox at various concentrations were calculated. Calibration curve was constructed by ploting lag time (sec) versus concentrations in mM Trolox on a graph paper, then sample were determined from the calibration curve. It was found that the curve was linear until at least 2 mM Trolox as shown in Figure 2.

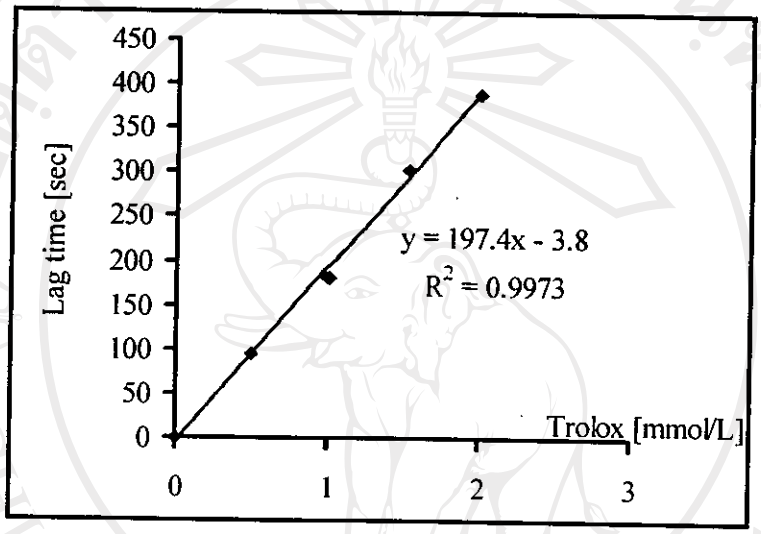


**Figure 2. Linearity curve of Trolox at 734 nm.**

The total antioxidant capacity of curcuminoids, α-tocopherol and ascorbic acid were determined by ABTS method using Trolox, a water-soluble vitamin E analogue as standard and thus the results were expressed as Trolox equivalent capacity. The levels of TAC in curcuminoids, α-tocopherol, and ascorbic acid were shown in Table 10. There were no significant difference of TAC between the curcuminoids and α- tocopherol (1.1±0.07 *vs.* 1.18± 0.06, $P < 0.05$), while ascorbic acid were significantly higher than those in curcuminoids and α-tocopherol (27.75±0.70 *vs.* 1.1±0.07 and 1.18±0.06, respectively, $P < 0.05$ ).

**Table 9.** Total antioxidant capacity of curcuminoids, α-tocopherol and ascorbic acid.

| Antioxidants (1 mg/mL) | Total antioxidant capacity (Trolox (mM), mean±SD, n=8) |
|---|---|
| Curcuminoids | 1.1±0.07 |
| α-Tocopherol | 1.18±0.06 |
| Ascorbic acid | 27.75±0.70* |

*$P < 0.05$ by Student's $t$ test

**3.3 Effect of curcuminoids, α-tocopherol and ascorbic acid on U937 cell cytotoxicity**

U937 cells at concentration of $1.0 \times 10^6$ cells/mL in completed RPMI-1640 medium were treated with curcuminoids, α-tocopherol and ascorbic acid at the final concentrations of 0, 10, 20, 30, 40, 50, 60, 80 and 100 μg/mL at 37° C, in 5% $CO_2$ for 24 and 48 hours. The cell viability was observed by trypan blue exclusion method and counted as the percent of total cells under light microscope.

The result found that curcuminoids at the concentration up to 30 μg/mL was not toxic to U937 cells and the cell viability was more than 90%. The cytotoxic effects of α-tocopherol and ascorbic acid were done in the same manner. The result also found that α-tocopherol up to 100 μg/mL and ascorbic acid up to 30 μg/mL were not toxic to U937 cells as shown in Figure 3.

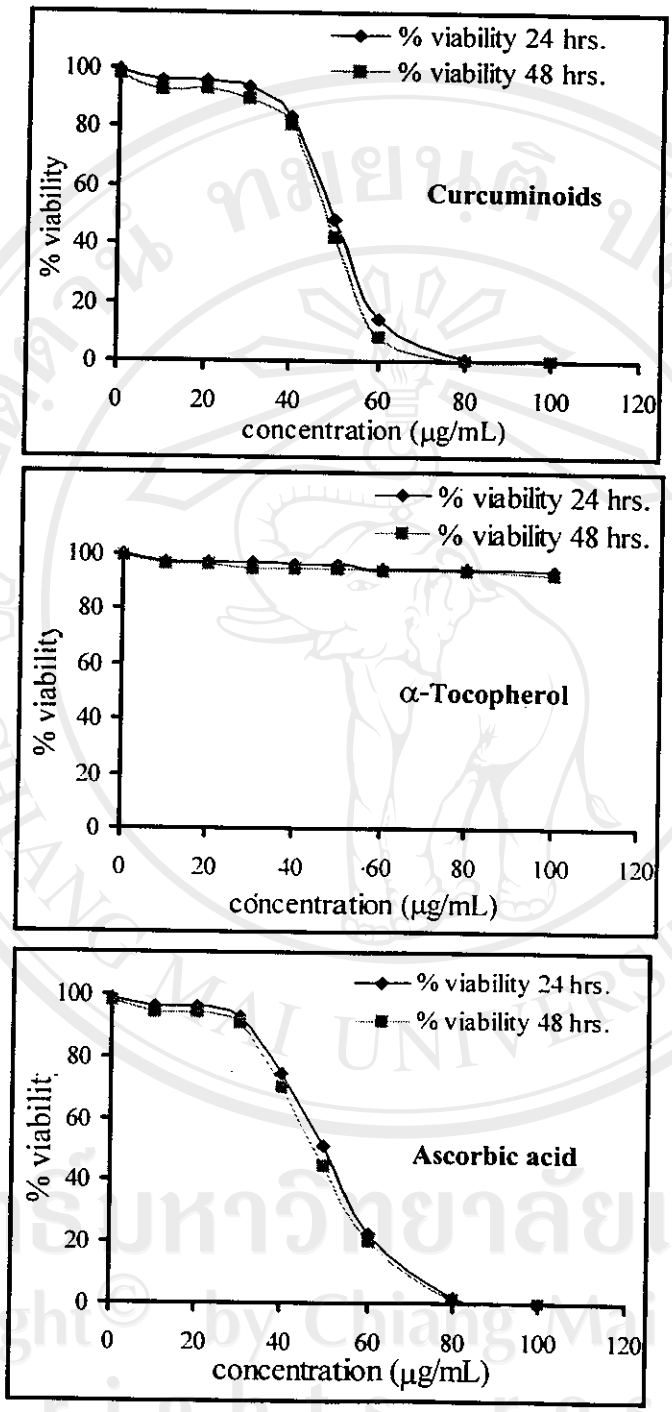


**Figure 3. Cytotoxicity effect of curcuminoids, α-tocopherol and ascorbic acid on U937 cell line measured by trypan blue exclusion method.**

### 3.4 LDL preparation and protein determination

#### 3.4.1 LDL separation from plasma

LDL was separated from other lipoproteins by single vertical discontinuous density gradient ultracentrifugation using a Beckman L-60 ultracentrifuge at 50,000 rpm, 10° C for 2.5 hours.

Three major lipoprotein fractions were separated as shown in Figure 4. After centrifugation, the three main lipoprotein fractions were collected. The uupper layer was VLDL with density range 1.014-1.016 g/mL, the middle layer was LDL with density range 1.020-1.062 g/mL and the bottom layer was HDL with density range 1.062-1.085 g/mL.

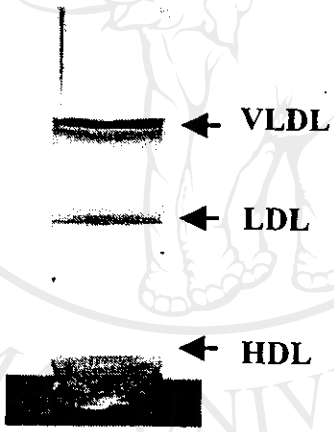


**Figure 4. VLDL, LDL and HDL fractions isolated by the single vertical spin discontinuous density gradient ultracentrifugation.**

### 3.4.2 Protein concentration of individual's LDL fraction

LDL protein concentration in plasma from healthy with normolipidemic and diabetic with hyperlipidemic groups determinated by the Lowry method were shown as mean±SD in Table 10. The LDL protein concentrations in diabetic group were significantly higher than those in healthy group (1.23±0.08 *vs.* 0.90±0.08, $P<0.05$).

**Table 10.** Protein concentrations (mg/mL, mean±SD) of LDL isolated from healthy with normolipidemic and diabetic with hyperlipidemic individuals.

| LDL isolation | LDL protein concentrations (mg/mL) | |
|---|---|---|
| | Healthy with normolipidemic group | Diabetic with hyperlipidemic group |
| 1 | 0.9 | 1.2 |
| 2 | 1.0 | 1.3 |
| 3 | 0.8 | 1.2 |
| 4 | 0.9 | 1.1 |
| 5 | 0.8 | 1.2 |
| 6 | 0.9 | 1.2 |
| 7 | 1.0 | 1.3 |
| 8 | 0.9 | 1.2 |
| 9 | 1.0 | 1.4 |
| 10 | 0.8 | 1.2 |
| Mean±SD | 0.90±0.08 | 1.23±0.08* |

*$P < 0.05$ by Student's *t* test

### 3.5 Evaluation of LDL and LDL oxidation

The LDL were diluted with PBS- free EDTA for a standard concentration of 200 μg/mL protein and initiated by the addition of freshly prepared $CuSO_4$ at final concentration of 20 μM at 37° C for 2 hours. LDL oxidation was evaluated in 3 different ways, the first was the mobility of ox-LDL on cellulose acetate electrophoresis, the second was the increase in absorbance at 234 nm for continuous monitoring of conjugated dienes formation and the third was the amount of malonaldehyde (MDA) formation as thiobarbituric acid reactive substance (TBARs) over 2 hours of incubation.

#### 3.5.1 Electrophoretic mobility

Cellulose acetate electrophoresis was used as the purity validation of the LDL fractions and LDL oxidation compared with plasma native LDL. The LDL electrophoretic mobility were shown in Figure 5. The plasma lipoproteins in plasma control were separated into 3 bands, LDL, VLDL and HDL (lanes 1). HDL was the fastest movement to anodic pole. The purity of LDL, VLDL and HDL after isolations were shown in lanes 2, 3 and 4, separately. All three isolations were slightly moved more anodic due to increased negative charge from isolation process. Oxidation of the LDL lipoprotein by $Cu^{2-}$, also increased its electrophoretic mobility and increased negative charge. LDL oxidation in lanes 7 and 8 showed a faster anodic electrophoretic mobility in cellulose acetate electrophoresis than native LDL band (lanes 5 and 6).

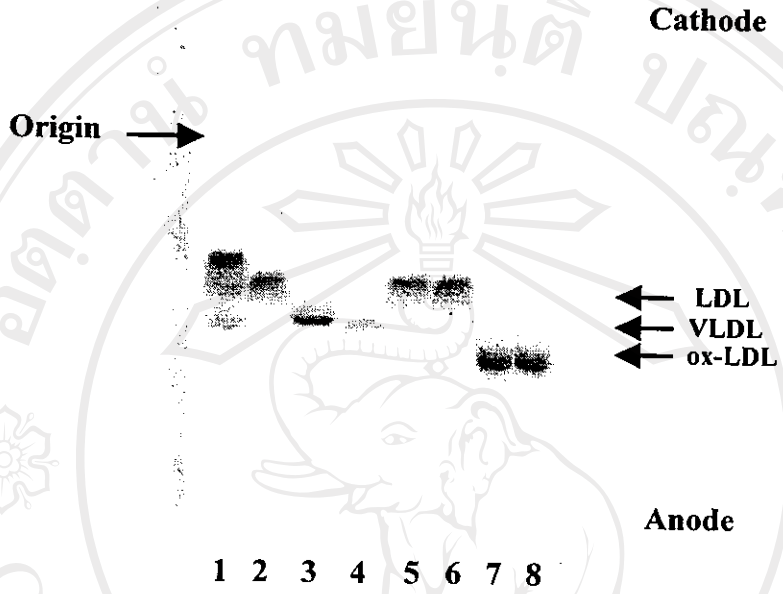


**Figure 5. Electrophoretic mobility of LDL and ox-LDL on cellulose acetate membrane.** Samples were electrophoresed at 180 volts for 20 minutes and then stained with Sudan black B. (1) plasma lipoproteins (2) LDL (3) VLDL (4) HDL (5) LDL from healthy person, (6) LDL from diabetics, (7) LDL from healthy person after incubated with $CuSO_4$, (8) LDL from diabetics after incubated with $CuSO_4$.

### 3.5.2 Conjugated diene formation

LDL oxidation determined as the production of hydroperoxides with conjugated double bonds (conjugated dienes) by continuously monitoring the change in absorbance at 234 nm. The $Cu^{2+}$-induced oxidation of LDL *in vitro* showed lag time of 55.5±2.79 minutes in healthy group and 47.8±2.29 minutes in diabetic group while the native LDL control showed the same absorbance.

### 3.5.3 Thiobarbituric acid reactive substances (TBARs) formation

LDL oxidation in healthy with normolipidemic and diabetic with hyperlipidemic groups were determined for MDA as TBARs formation. The MDA concentrations were increased in ox-LDL compared with native LDL (16.2±1.13 *vs.* 4.2±0.63 μM in healthy group and 19.0±0.81 *vs.* 6.70±0.94 μM in diabetic group).

## 3.6 The oxidative susceptibility of LDL in healthy with normolipidemic and diabetic with hyperlipidemic groups

The oxidative susceptibility of LDL in healthy and diabetic groups were evaluated in 3 different ways as follows.

### 3.6.1 Cellular LDL uptake by U937 cells

U937 cells were incubated with the final concentration of 100 μg/mL LDL and ox-LDL in culture medium. Incubation were carried out at 37° C in a humidified atmosphere containing 5% $CO_2$ for 24 hours. Cells were observed under simple light microscope, original magnification x 100 after stained with oil red O and Mayer's hematoxylin.

The oxidative susceptibility of LDL in healthy and diabetic groups were determined the LDL uptake by LDL receptor on U937 cells. The susceptibility of ox-LDL *in vitro* was higher significantly in diabetic than in healthy group. The percentage of cellular LDL uptake in diabetic was significantly higher than in healthy group (85.6±1.71 *vs.* 76.5±2.17 %, $P<.005$). The percentage of cellular LDL uptake in two groups were shown in Figure 6.

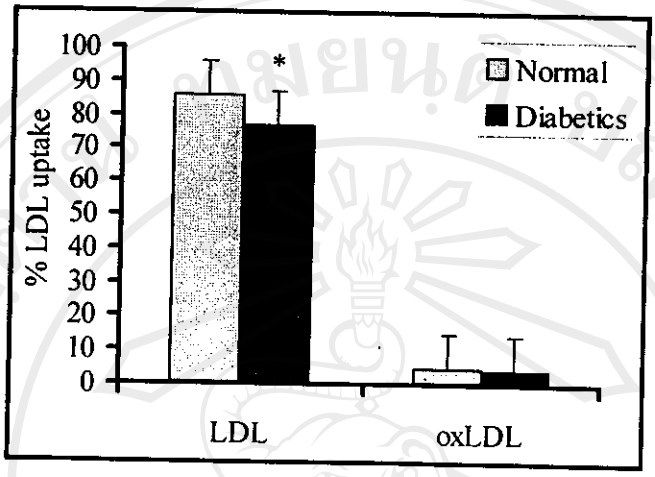


**Figure 6. Oxidative susceptibility of LDL determined by U937 cellular LDL uptake.** U937 cells were incubated with LDL and ox-LDL from both groups. The percentage of cellular LDL uptake were observed under simple light microscope, after stained with oil red O and Mayer's hematoxylin.

*$P < 0.05$ by Student's $t$ test

### 3.6.2 Conjugated dienes formation

The comparison the oxidative susceptibility of LDL between healthy and diabetic groups were determined for the production of hydroperoxides with conjugated dienes by continuously monitoring the change in absorbance at 234 nm. The kinetic curves of conjugate dienes and lag times in both groups were shown in Figure 7. The susceptibility of LDL oxidation *in vitro* was higher significantly in diabetic group than healthy group. The lag time of conjugated dienes in healthy group was longer than diabetic group (55.5±2.79 *vs.* 47.8±2.29 minutes, $P$ <.005).

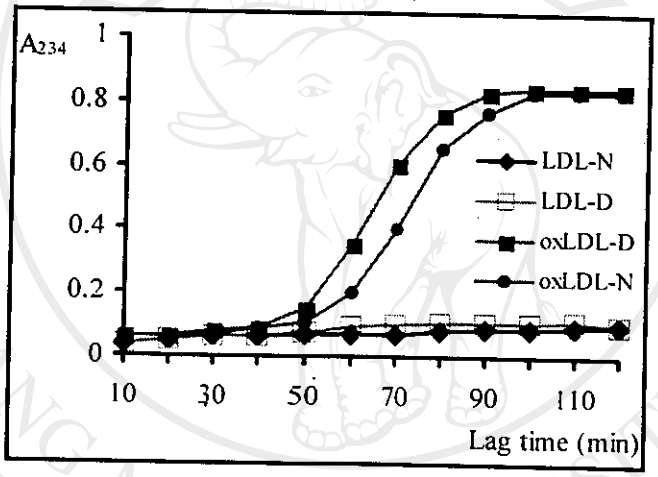


**Figure 7. Oxidative susceptibility of LDL determined by conjugated dienes formation.** The oxidative susceptibility of LDL oxidation was analyzed in healthy and diabetic group at 234 nm. LDL-N = LDL in healthy group, LDL-D = LDL in diabetic group, oxLDL-D = LDL with $CuSO_4$ in diabetic group, and oxLDL-N = LDL with $CuSO_4$ in healthy group.

### 3.6.3 Thiobarbituric acid reactive substances (TBARs) formation

The oxidative susceptibility of LDL in healthy and diabetic groups were compared by determination of MDA concentrations as TBARs reaction. The result found that the susceptibility of LDL oxidation *in vitro* was higher significantly in diabetic group than healthy group as shown in Figure 8. The MDA concentration of LDL and ox-LDL in healthy group were significantly lower than diabetic group (4.2±0.63 *vs.* 16.2±1.13 and 6.70±0.94 *vs.* 19.0±0.81 μM, respectively, $P<0.05$). The increasing of MDA concentration was lower in healthy group than diabetic group (12.0±0.54 *vs.* 13.0±0.62 μM).

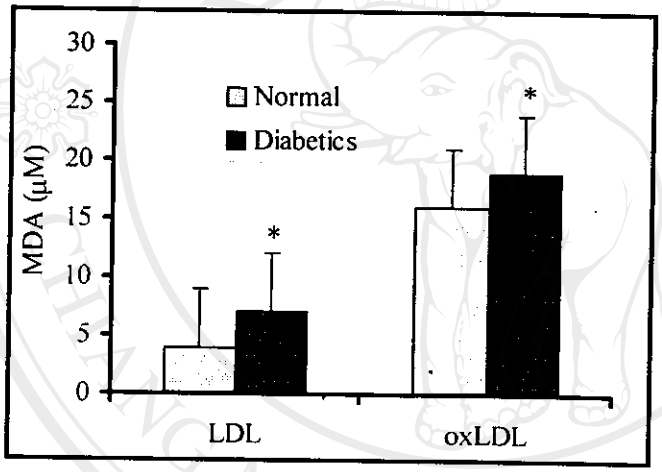


**Figure 8. Oxidative susceptibility of LDL determined by TBARs formation.** The MDA concentration of LDL and ox-LDL in healthy and diabetic groups were compared by TBARs reaction.

*$P < 0.05$ by Student's *t* test

### 3.7 The effect of curcuminoids on inhibition $Cu^{2+}$-induced LDL oxidation

#### 3.7.1 Comparison between healthy and diabetic groups

The effect of curcuminoids on inhibition LDL oxidation in healthy and diabetic groups were evaluated by 3 different ways as follows.

##### 3.7.1.1 Cellular LDL uptake by U937 cells

To study the effect of curcuminoids on inhibition of $Cu^{2+}$-induced LDL oxidation, 20 μM $CuSO_4$ was added in 200 μg/mL protein of LDL in the presence or absence of 30 μg/mL curcuminoids at the final concentration. The inhibition of LDL oxidation at 37° C for 2 hours were determined by U937 cellular LDL uptake from healthy and diabetic groups.

The LDL oxidation was efficiently inhibited by curcuminoids. The cellular LDL uptake were significantly higher than in the absence of curcuminoids in healthy and diabetic groups (71.9±0.99 *vs.* 5.1±0.73, and 65.8±1.39 *vs.* 4.00±0.81 %, respectively, $P<0.05$). The percentages of cellular LDL uptake were shown in Figure 9.

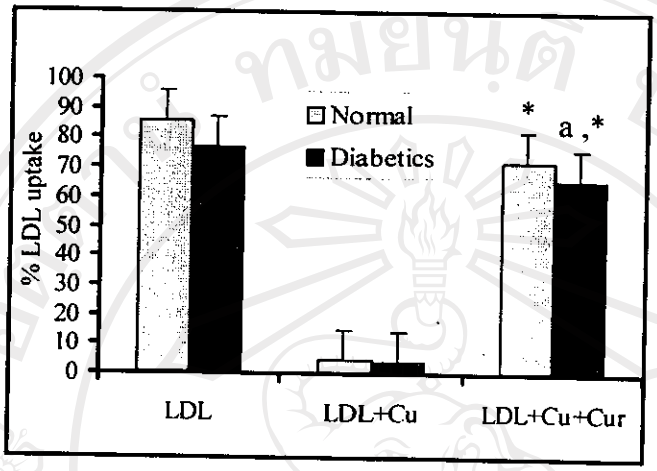


**Figure 9. Effect of curcuminoids on inhibition of $Cu^{2+}$-induced LDL oxidation determined by U937 cellular LDL uptake.** Significant differences between LDL with $CuSO_4$ and LDL with $CuSO_4$ in presence of curcuminoids are indicated by $^*P < 0.05$. Significant difference between LDL with $CuSO_4$ in healthy and diabetic groups is indicated by $^aP < 0.05$.

(Cur = Curcuminoids and Cu = $CuSO_4$)

### 3.7.1.2 Conjugated diene formation

The effect of curcuminoids on $Cu^{2+}$-induced oxidation of LDL was determined as the production of hydroperoxides with conjugated dienes by continuously monitoring the change in absorbance at 234 nm. The presence of 30 μg/mL curcuminoids was efficiently inhibited LDL oxidation by increasing lag time in both healthy and diabetic groups (91.2±1.39 *vs.* 55.5±2.79 minutes and 81.8±1.39 *vs.* 47.8±2.29 minutes, respectively, $P<0.05$) as shown in Figure 10.

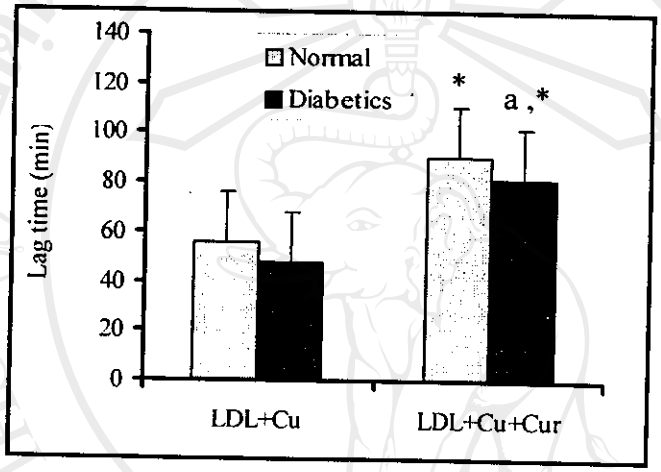


**Figure 10. Effect of curcuminoids on inhibition of $Cu^{2+}$-induced LDL oxidation determined by conjugated diene formation.** Significant differences between LDL with $CuSO_4$ and LDL with $CuSO_4$ in presence of curcuminoids are indicated by $^*P < 0.05$. Significant difference between LDL with $CuSO_4$ in healthy and diabetic groups is indicated by $^aP < 0.05$.
(Cur = Curcuminoids and Cu = $CuSO_4$)

### 3.7.1.3 Thiobarbituric acid reactive substances (TBARs) formation

The effect of curcuminoids on $Cu^{2+}$-induced oxidation of LDL determined by MDA formation as TBARS. The presence of 30 μg/mL curcuminoids was efficiently inhibited LDL oxidation as lower MDA concentration was observed in both healthy and diabetic groups (6.9± 0.73 *vs.* 16.2±1.13 and 11.1±0.87 *vs.* 19.0±0.81 μM, respectively, $P<0.05$) as shown in Figure 11.

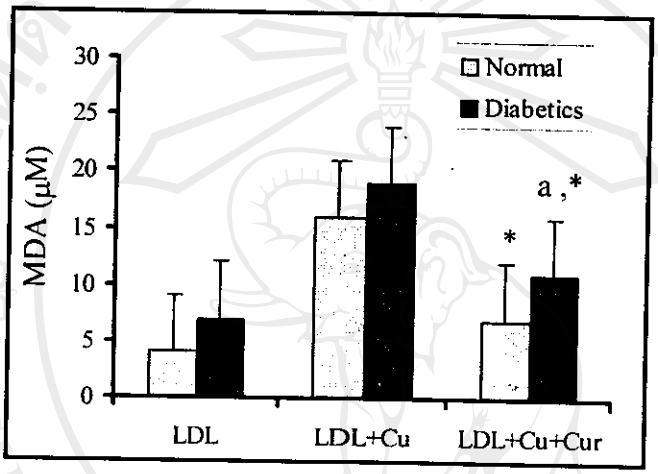


**Figure 11. Effect of curcuminoids on $Cu^{2+}$-induced LDL oxidation determined by TBARs formation.** Significant differences between LDL with $CuSO_4$ and LDL with $CuSO_4$ in presence of curcuminoids are indicated by $^*P < 0.05$. Significant difference between LDL with $CuSO_4$ in healthy and diabetic groups is indicated by $^aP < 0.05$.

(Cur = Curcuminoids and Cu = $CuSO_4$)

### 3.7.2 Comparison with α-tocopherol and ascorbic acid

The effect of curcuminoids compare to α-tocopherol and ascorbic acid on inhibition LDL oxidation were evaluated in 3 different ways as follows.

#### 3.7.2.1 Cellular LDL uptake by U937 cells

LDL were diluted in PBS to containing 200 μg/mL of protein and 20 μM $CuSO_4$ in the presence or absence of curcuminoids, α-tocopherol and ascorbic acid at the final concentrations of 30 μg/mL at 37° C for 2 hours. The effect of curcuminoids on inhibition of LDL oxidation were determined for cellular LDL uptake by U937 cells and compare with α-tocopherol and ascorbic acid in healthy and diabetic groups.

The percentage of cellular LDL uptake as shown in Figure 12. Curcuminoids, α-tocopherol and ascorbic acid could retard the LDL oxidation and increase cellular LDL uptake in both healthy and diabetic groups (71.9±0.99, 76.0±2.00, 72.5±1.71 *vs.* 5.1±0.73 and 65.8±1.39, 70.2±1.54, 67.0±2.53 *vs.* 4.00±0.81 %, respectively, $P<0.05$). In the presence of curcuminoids, α-tocopherol and ascorbic acid showed the percentage inhibition of LDL oxidation decreased by approximately 83%, 88%, 84% and 77%, 82%, 78%, respectively in both healthy and diabetic groups (Figure 12). The effect of α-tocopherol on $Cu^{2+}$-induced oxidation of LDL was significantly greater (higher cellular uptake LDL) than curcuminoids and ascorbic acid in both groups (76.0±2.00 *vs.* 71.9±0.99, 76.0±2.00 *vs.* 72.5±1.71 and 70.2±1.54 *vs.* 65.8±1.39, 70.2± 1.54 *vs.* 67.0±2.53 %, respectively, $P<0.05$) while curcuminoids has no significant different effect on cellular LDL uptake than ascorbic acid in both groups (71.9±0.99 *vs.* 72.5±1.71 and 65.8± 1.39 *vs.* 67.0±2.53 %, respectively, $P>0.05$). The percentage inhibition of LDL oxidation in the presence of α-tocopherol was significantly higher than curcuminoids and ascorbic acid in both healthy and diabetic groups (88.19±4.23 *vs.* 82.98±5.10, 88.19±4.23 *vs.* 83.72±3.67 and 82.23± 5.21 *vs.* 77.10±4.62, 82.23±5.21 *vs.* 78.26±3.94 %, respectively, $P<.005$) while the percentage inhibition of LDL oxidation in the presence of curcuminoids was no significant different from ascorbic acid in both groups (82.98±5.10 *vs.* 83.72±3.67 and 77.10±4.62 *vs.* 78.26±3.94 %, respectively, $P>0.05$).

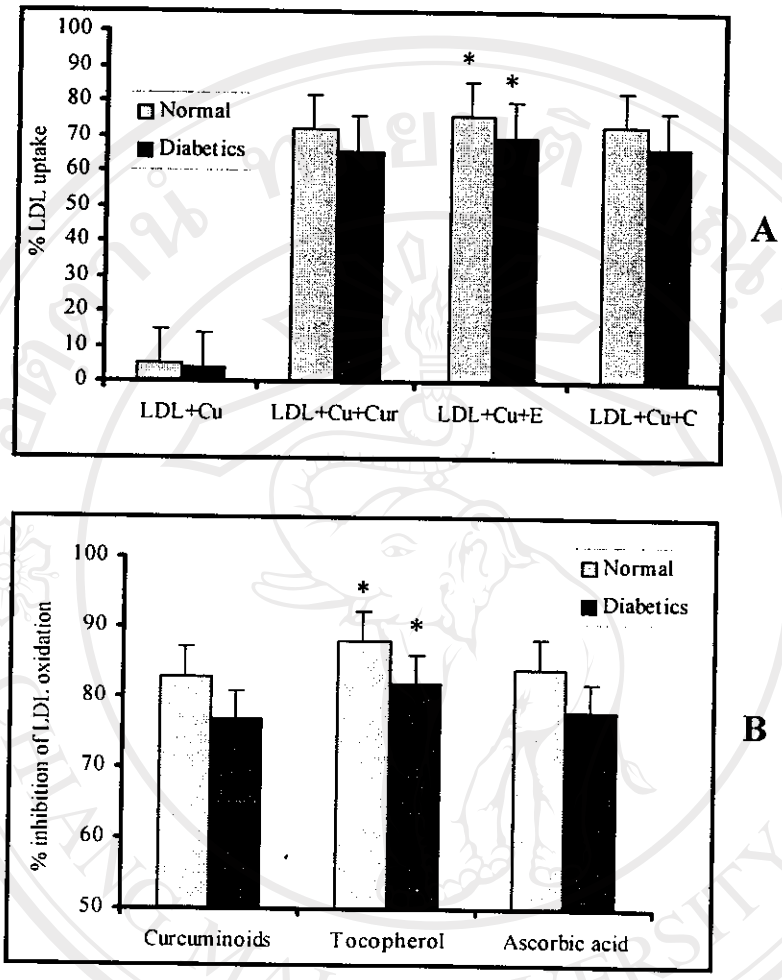


**Figure 12. Comparison the effect of curcuminoids, α-tocopherol and ascorbic acid on $Cu^{2+}$-induced LDL oxidation determined by U937 cellular LDL uptake.** **A** = percentage of cellular LDL uptake in healthy and diabetic groups. **B** = percentage inhibition of LDL oxidation by antioxidants in healthy and diabetic groups. Significant differences between LDL with $CuSO_4$ in presence of curcuminoids with α-tocopherol or ascorbic acid are indicated by $*P < 0.05$.
(Cur = Curcuminoids, E = α-Tocopherol, C = Ascorbic acid and Cu = $CuSO_4$)

**3.7.2.2 Conjugated diene formation**

The effect of curcuminoids, α-tocopherol and ascorbic acid on $Cu^{2+}$-induced oxidation of LDL were determined as the production of hydroperoxides with conjugated dienes was shown in Figure 13. The presence of 30 mg/ml of Curcuminoids, α-tocopherol and ascorbic acid retarded the LDL oxidation (increased the lag time) and significantly increase the lag time than in the absence of antioxidants in healthy and diabetic groups (91.2±1.39 *vs.* 55.5±2.79, 95.0±1.82 *vs.* 55.5±2.79, 92.8±1.75 *vs.* 55.5±2.79 minutes and 81.8±1.39 *vs.* 47.8±2.29, 84.8±2.25 *vs.* 47.8± 2.29, 83.4±1.50 *vs.* 47.8±2.29 minutes, respectively, $P<0.05$). The presence of curcuminoids, α-tocopherol and ascorbic acid was significantly increased lag time by approximately 66%, 71%, 67% and 63%, 67%, 64%, respectively than in the absence of antioxidants in both groups. The effect of α-tocopherol on $Cu^{2+}$-induced oxidation of LDL was significantly greater (longer lag time) than curcuminoids and ascorbic acid in both healthy and diabetic groups (95.0±1.82 *vs.* 91.2±1.39, 95.0±1.82 *vs.* 92.8±1.75 and 84.8±2.25 *vs.* 81.8±1.39, 84.8±2.25 *vs.* 83.4±1.50 minutes, respectively, $P<0.05$), while the effect of curcuminoids was the same as ascorbic acid in both healthy and diabetic groups (91.2±1.39 *vs.* 92.8±1.75 and 81.8±1.39 *vs.* 83.4±1.50 minutes, respectively, $P>0.05$). The percentage increased lag time of LDL oxidation in the presence of α-tocopherol was significantly higher than curcuminoids and ascorbic acid in both healthy and diabetic groups (71.20±2.43 *vs.* 66.12±2.24, 71.20±2.43 *vs.* 67.20±3.01 and 67.17±2.79 *vs.* 63.04±2.46, 67.17±2.79 *vs.* 64.14±2.19 %, respectively, $P<0.05$) while the percentage increased lag time of LDL oxidation in the presence of curcuminoids was the same as ascorbic acid in both healthy and diabetic groups (66.12±2.24 *vs.* 67.20±3.01 and 63.04±2.46 *vs.* 64.14±2.19 %, respectively, $P>0.05$).

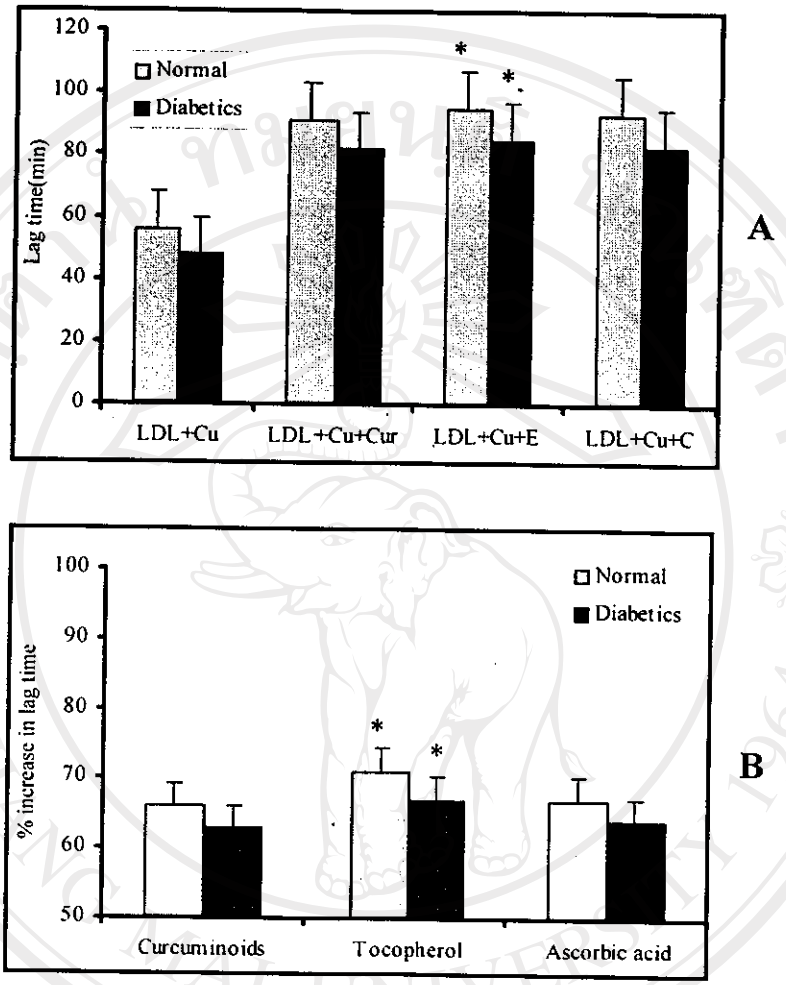


**Figure 13. Comparison the effect of curcuminoids, α-tocopherol and ascorbic acid on $Cu^{2+}$-induced LDL oxidation monitored at 234 nm.** **A** = lag time values in healthy and diabetic groups. **B** = percentage inhibition of LDL oxidation by antioxidant in healthy and diabetic groups. Significant differences between LDL with $CuSO_4$ in presence of curcuminoids with α-tocopherol or ascorbic acid are indicated by $*P < 0.05$.

(Cur = Curcuminoids, E = α-Tocopherol, C = Ascorbic acid and Cu = $CuSO_4$)

### 3.7.2.3 Thiobarbituric acid reactive substances (TBARs) formation

The effect of curcuminoids, α-tocopherol and ascorbic acid on $Cu^{2+}$-induced oxidation of LDL were determined as MDA concentration by TBARS as shown in Figure 14. The presence of 30 μg/mL curcuminoids, α-tocopherol and ascorbic acid retarded the LDL oxidation making significantly lower MDA concentrations than in the absence of the antioxidants in both healthy and diabetic groups (6.9±0.73 *vs.* 16.2±1.13, 6.0±0.81 *vs.* 16.2±1.13, 6.7±0.67 *vs.* 16.2±1.13 and 11.1±0.87 *vs.* 19.0±0.81, 10.0±0.94 *vs.* 19.0±0.81, 11.0±0.81 *vs.* 19.0±0.81 μM, respectively, $P$ <0.05). The percentage inhibition of LDL oxidation in the presence of curcuminoids, α-tocopherol and ascorbic acid were approximately 78%, 85%, 78% and 66%, 74%, 66%, respectively in both groups. The inhibition effect of α-tocopherol on $Cu^{2+}$-induced oxidation of LDL was significantly greater (lower MDA concentration) than curcuminoids and ascorbic acid in both healthy and diabetic groups (6.0±0.81 *vs.* 6.9±0.73, 6.0±0.81 *vs.* 6.7±0.67 and 10.0±0.94 *vs.* 11.1±0.87, 10.0±0.94 *vs.* 11.0±0.81 μM, respectively, $P$<0.05) while the effect of curcuminoids was the same as ascorbic acid in both healthy and diabetic groups (6.9±0.73 *vs.* 6.7±0.67 and 11.1±0.87 *vs.* 11.0±0.81 μM, respectively, $P$>0.05). The percentage inhibition of LDL oxidation in the presence of α-tocopherol was significantly higher than curcuminoids and ascorbic acid in healthy and diabetic groups (85.20±6.42 *vs.* 78.30±7.39, 85.20±6.42 *vs.* 78.10± 8.19 and 74.49±9.79 *vs.* 66.10±7.29, 74.49±9.79 *vs* 66.00±7.78 %, respectively, $P$<.005) while the percentage inhibition of LDL oxidation in the presence of curcuminoids was the same as ascorbic acid in both healthy and diabetic groups (78.30±7.39 *vs.* 78.10±8.19 and 66.10±7.29 *vs.* 66.00±7.78 %, respectively, $P$>0.05).

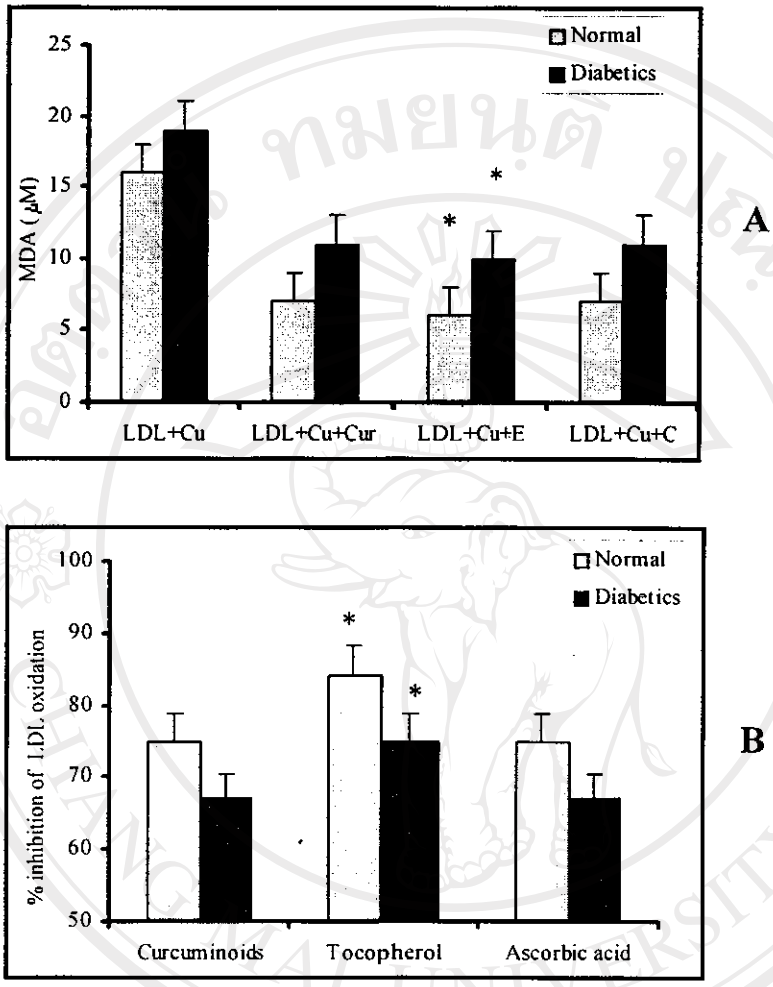


**Figure 14. Comparison the effect of curcuminoids, α-tocopherol and ascorbic acid on the $Cu^{2+}$-induced LDL oxidation determined by TBARs formation.** **A** = MDA concentration values in healthy and diabetic group. **B** = percentage inhibition of LDL oxidation by antioxidant in healthy and diabetic groups. Significant differences between LDL with $CuSO_4$ in presence of curcuminoids with α-tocopherol or ascorbic acid are indicated by $^*P < 0.05$.

(Cur = Curcuminoids, E = α-Tocopherol, C = Ascorbic acid and Cu = $CuSO_4$)

**3.8 The effect of curcuminoids on ox-LDL determined cellular LDL uptake by U937 cells**

**2.8.1 Comparison between healthy and diabetic groups**

Oxidized LDL from healthy and diabetic groups were diluted with PBS containing 200 μg/mL of protein and curcuminoids at the final concentration of 30 μg/mL was added, then the mixture was incubated at 37° C for 2 hours. The effect of curcuminoids on ox-LDL was determined by U937 cellular LDL uptake.

The percentage of cellular LDL uptake were shown in Figure 15, curcuminoids had no effect on ox-LDL. The presence of 30 μg/mL of curcuminoids could not reverse the ox-LDL to native LDL and there was no significant different in U937 cellular LDL uptake from the one with the absence of curcuminoids in both groups (6.2±0.0.63 *vs.* 5.1±0.73 and 5.6±0.68 *vs.* 4.6±0.81 %, respectively, $P>0.05$).

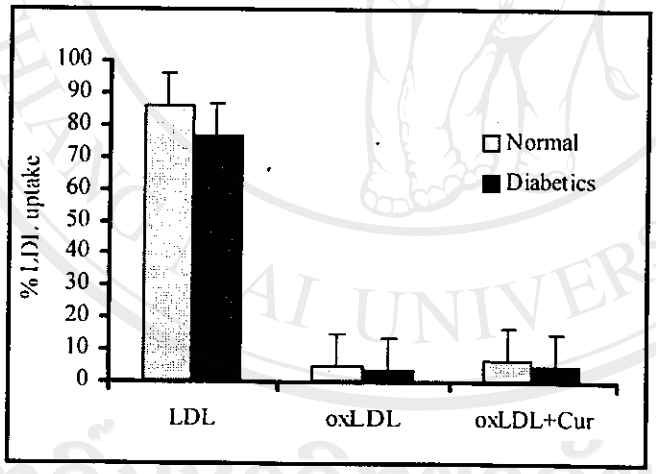


**Figure 15. Effect of curcuminoids on ox-LDL determined by U937 cellular LDL uptake.** Curcuminoids was added to 200 μg/mL ox-LDL at the final concentration of 30 μg/mL and then incubated with U937 cells. (Cur = Curcuminoids)

### 3.8.2 Comparison with α-tocopherol and ascorbic acid

Oxidized LDL were diluted with PBS containing 200 μg/mL of protein and curcuminoids, α-tocopherol or ascorbic acid at the final concentration of 30 μg/mL were added after incubation at 37° C for 2 hours. The effect of curcuminoids, α-tocopherol and ascorbic acid on ox-LDL was determined by U937 cellular LDL uptake.

The effects of Curcuminoids, α-tocopherol and ascorbic acid on ox-LDL in healthy and diabetic groups were compared by U937 cellular uptake of LDL as shown in Figure 16. All antioxidants studied did not affect on ox-LDL. The presence of 30 μg/mL of curcuminoids, α-tocopherol and ascorbic acid could not reverse the ox-LDL to native LDL and the cellular LDL uptake were not significant different from in the absence of antioxidants in both healthy and diabetic groups (6.2±0.0.63, 6.0±0.81, 6.1±0.87 *vs.* 5.1±0.73 and 5.6±0.68, 5.5±0.73, 5.6±0.73 *vs.* 4.6±0.81 %, respectively, $P>0.05$). The effect of curcuminoids on ox-LDL was the same as α-tocopherol and ascorbic acid in both healthy and diabetic groups.

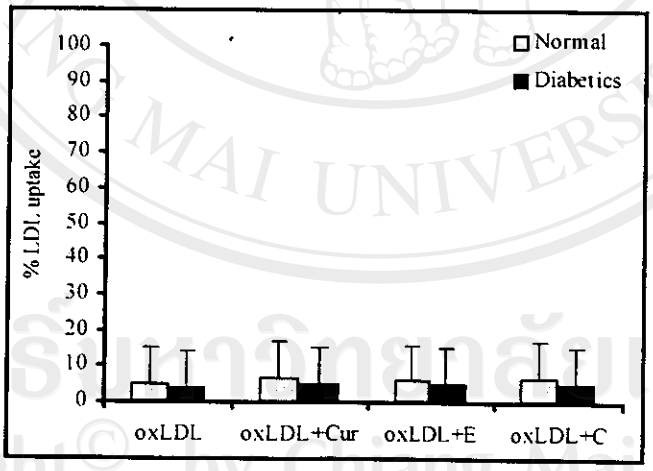


**Figure 16. Comparison the effect of curcuminoids, α-tocopherol and ascorbic acid on ox-LDL determined by U937 cellular LDL uptake.** Three antioxidants were added to 200 μg/mL ox-LDL at the final concentration of 30 μg/mL and then incubated with U937 cells.
(Cur = Curcuminoids, E = α-Tocopherol, C = Ascorbic acid and Cu = $CuSO_4$)