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ORIGINAL ARTICLE

# EFFECTS OF YOUNG BARLEY LEAF EXTRACT AND ANTIOXIDATIVE VITAMINS ON LDL OXIDATION AND FREE RADICAL SCAVENGING ACTIVITIES IN TYPE 2 DIABETES

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**SUMMARY** - **Background:** The effects of supplementation of young barley leaf extract (BL) and/or antioxidative vitamins C and E on different low-density lipoprotein (LDL) subfractions susceptibility to oxidation and free radical scavenging activities in patients with type 2 diabetes were evaluated.
**Methods:** Thirty-six type 2 diabetic patients were enrolled in this study. The subjects received one of the following supplements daily for 4 weeks: 15 g BL, 200 mg vitamin C and 200 mg vitamin E (CE), or BL plus CE (BL + CE).
**Results:** The lucigenin-chemiluminescence (CL) and luminol-CL levels in blood were significantly reduced in all groups. Vitamin E content of LDL subfractions increased significantly following supplements, especially for BL + CE group. The percent increase of lag times in the BL + CE was significantly higher than those in the BL or CE group. The antioxidative effect of BL + CE was the greatest for small, dense LDL (Sd-LDL) with further increases in percentage of lag times 4 folds compared to BL alone.
**Conclusion:** Our results indicate that supplementation with BL may help to scavenge oxygen free radicals, save the LDL-vitamin E content, and inhibit LDL oxidation. Furthermore, the addition of vitamins C and E to BL can inhibit the Sd-LDL oxidation more effectively, which may protect against vascular diseases in type 2 diabetic patients.

*Key-words:* Barley leaf extract, vitamins C and E, LDL oxidation, free radical scavenging activity, type 2 diabetes.

**RÉSUMÉ** - **Effets d'extraits de jeunes feuilles d'orge et de vitamines antioxydantes sur l'oxydation des LDL et l'activité scavenging de radicaux libres au cours du diabète de type 2.**
**Contexte :** Les effets de la supplémentation par de l'extrait de jeunes feuilles d'orge (FO) et/ou par des vitamines antioxydantes C et E sur la susceptibilité de différentes sous-fractions des lipoprotéines de basse densité (LDL) à l'oxydation et sur les activité scavenging de radicaux libres ont été évalués chez des diabétiques de type 2.
**Méthodes :** Trente-six patients diabétiques de type 2 ont été inclus dans cette étude. Les sujets ont reçu un des suppléments suivants tous les jours pendant 4 semaines : 15 g FO, 200 mg vitamine C et 200 mg vitamin E (CE), ou FO plus CE (FO + CE).
**Résultats :** La chemiluminescence- lucigenin (CL) et les taux de luminol-CL dans les sang étaient significativement diminués dans tous les groupes. Le contenu en vitamine E des sous-fractions LDL a augmenté significativement sous supplémentation, notamment dans le groupe FO + CE. Le pourcentage d'augmentation du lag time dans le groupe FO + CE était significativement plus élevé que dans le groupe FO ou CE. L'effet antioxydant de FO + CE était le plus marqué pour les LDL petites et denses avec une augmentation supplémentaire du pourcentage de lagtime d'un facteur 4 par rapport au groupe FO seul.
**Conclusion :** Nos résultats indiquent qu'une supplémentation par FO peut aider au scavenging des radicaux libres, à épargner le contenue des LDL en vitamine E, et à inhiber l'oxydation des LDL. En outre, l'ajout de vitamines C et E au FO peut inhiber l'oxydation des LDL petites et denses de façon plus efficace, ce qui peut protéger contre les maladies vasculaires du diabétique de type 2.

*Mots-clés :* extrait de feuille d'orge, vitamines C et E, oxuydation des LDL, scavenging des radicaux libres, diabète de type 2.

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Diabetics have a higher risk of developing coronary artery disease and peripheral atherosclerosis than the general population. Patients with type 2 diabetes often have elevated levels of triacylglycerol (TG) and small, dense LDL (Sd-LDL) along with lower levels of HDL cholesterol [1]. The particles of Sd-LDL (d = 1.040-1.054 g/ml) are more susceptible to oxidation than larger, buoyant LDL (B-LDL) [2] because both their entry into [3] and retention within [4] the artery walls are greater than those of larger lipoprotein particles. High blood sugar is commonly associated with increased oxidative changes in LDL [5]. Moreover, the metabolic changes caused by hyperglycemia include increased polyol pathway flux, elevated oxygen free radical formation, and advanced glycosylation [6]. All of these factors appear to accelerate red blood cell hemolysis and plasma LDL oxidation [7]. In some previous studies demonstrated that LDL from type 2 diabetic patients was more susceptible to oxidation, possibly as a result of lower levels of α-tocopherol in LDL than normals [8], but the result is still controversial [9-11]. Supplementation with vitamin E may lead to more than a three-fold increase in the lag time of B-LDL than that of Sd-LDL. Thus, Sd-LDL may procure less protection against oxidation from antioxidant supplementation than B-LDL [12]. This condition may contribute to an increased risk of coronary heart disease and other vascular diseases for type 2 diabetic patients.

In one survey, increases in dietary and plasma content of antioxidants, such as vitamins C and/or E, were associated with a lower risk of cardiovascular diseases [13]. Other studies demonstrated that supplementation with a combination of vitamins C and E was associated with greater reductions in the risk of cardiovascular diseases than supplementation with vitamins C or E alone [14].

Young barley leaf extract (BL) is a good natural source of vitamins and minerals. It contains polyphenolic compounds and has been found to have antioxidant activity in a lipid peroxidation system [15].

The present study was conducted to assess the effects of supplementation with BL, vitamins C and E, or with the combination of BL plus vitamins C and E on plasma lipids. The susceptibilities of various LDL fractions to oxidation and free radical scavenging activities in type 2 diabetic patients were studied.

## METHODS AND MATERIALS

### Subjects

Thirty-six patients with type 2 diabetes (fasting plasma glucose > 7.7 mmol/L, $HbA_{1c}$ > 6%) were randomly selected from the diabetic clinics of the China Medical College Hospital, Taichung, Taiwan. Patients were excluded from the study if they had diabetic complications, such as proteinuria (protein in urines), abnormal eye fundus, neuropathy, or history of liver diseases. None of the subjects had acute medical problems and none took lipid-lowering medications, vitamins or other antioxidants supplementation. Hyperglycemia was controlled using diet (containing 10-15% protein, 30% fat and 55-60% carbohydrates) and oral agents (glipizide or glyburide). No changes in the management of hyperglycemia were incorporated during the study. All subjects were volunteers and gave written, informed consent. The protocol was approved by the Academic Research Committee of China Medical College.

The 36 subjects were randomly divided into three groups. The first group of subjects received 15 g/d BL (YH Products Co., Oxnard, California, U.S.A.). The second group received 200 mg/d vitamin C and 200 mg/d vitamin E (Jen Sheng Pharmaceutical Co., Taichung, Taiwan). The third group received 15 g/d BL plus 200 mg/d vitamin C and 200 mg/d vitamin E. BL contained antioxidants, including β-carotene (24.962 IU/100 g), vitamin C (100 mg/100g) vitamin E (10.8 mg α-tocopherol/100 g) (analyzed by YH Products Co.,) and phenolic compounds (291.6 mg gallic acid/100 g). The three-day dietary record and diet instructions, including requests to not take vitamins or eat out during the period of experiment, were given to the patients when they first entered the program. The treatment started at week 0, about four weeks after they entered the dietary program, and ended at week 4.

### Determination of total phenolics content

The total content of phenolics was determined according to the method of Taga *et al.* in 1984 [16] and calculated using gallic acid (Sigma Chemical Co., Montana, USA) as a standard. Ten grams BL was extracted by 200 mL methanol for more than 2 hours in an extraction flask, then filtered. The methanol filtrate was evaporated and dissolved in a methanol/water mixture (60:40, 0.3% HCl), to bring the final concentration to 50 mg/mL. The resulting solution (100 μL) was added to 2 mL of 2% $Na_2CO_3$. After 2 min, 100 mL Folin-Ciocalteu reagent (50%) was added to the mixture, which was then left for 30 min. Absorbance was measured at 750 nm using a spectrophotometer. Results were expressed as milligrams per gram of gallic acid/100 g BL powder.

### Blood collection and analyses

Venous blood samples were collected from patients after a 12-hour overnight fasting when they first entered, the treatment started (week 0) and at the end of the four-week supplementation (week 4). The blood was centrifuged at 1.000 × g for 15 min at 4 (C to obtain plasma. Plasma TG concentrations were as-

sayed by an enzymatic GPO-PAP test [17] using commercial enzymatic kit (Merk Ltd., Darmstadt, FRG). Plasma and lipoprotein cholesterol concentrations were measured by a commercial enzymatic CHOD-PAP test kit [18] (Merk Ltd., Darmstadt, FRG). Fasting glucose concentrations were determined by a GDH enzymatic UV test, using a commercial kit (Roche Product Co., Ontario, Canada). Hemoglobin A1c ($HbA_{1c}$) was measured using high performance liquid chromatography (HLC-723 GHb Model II Analyzer) [19]. The reference range for $HbA_{1c}$ was 3.4% to 5.8%.

### Lag phase of LDL oxidation

B-LDL (d = 1.022-1.032 g/ml) and Sd-LDL (d = 1.04-1.054 g/ml) were isolated using preparative micro-ultracentrifugation in NaBr-NaCl solutions as previously described [20]. LDL oxidation was undertaken after overnight dialysis against phosphate-buffered saline (PBS) within 48 hours of isolation. Oxidation of LDL was determined as the production of conjugated dienes by continuously monitoring the changes in absorbance at 234 nm. The formation of conjugated dienes was measured by incubating 50 µg LDL with 5 µmol/L copper sulfate ($Cu^{++}$) in 1 mL PBS at 37°C. Lag times were determined from the time of the formation of conjugated diene during $Cu^{++}$-mediated oxidation of LDL [21]. The length of the lag phase was defined as the time (minutes) to the intercept of the tangent of the absorbance curve in the propagation phase with baseline. $\Delta A_{234\,nm}$ was defined as the maximum increase in absorbance. Propagation rate was expressed as the slope of the tangent (change in absorbance/min).

### Lipoprotein vitamin E

Vitamin E was measured using high performance liquid chromatography (HPLC) according to the method of Kaplan *et al.* [22]. To determine the vitamin E content of LDL, a 200 µL aliquot of LDL was taken and 5 µg a-tocopherol acetate was added as an internal standard. The LDL were extracted with ethanol and dried under nitrogen. The extract was reconstituted in a mobile phase which consisted of acetonnitrile: tetrahydrafuran (70:30, V:V) and run at a flow rate of 1 mL/min. The chromatography analysis was performed with a LiChroCART 250-4 Purospher RP-18 (5 µm) column (Merk Ltd., Darmstadt, FRG) and monitored at 292 nm in an UV detector (LC-400, Hitachi, Japan). Calculations were determined from the standard curve of peak area ratios of the sample to internal standards.

### Measurement of free radicals *in vivo*

A lucigenin and luminol amplified chemiluminescence (CL) were used to quantify superoxide radicals and oxygen free radicals in peripheral blood. The method for measuring luminol-CL or lucigenin-CL was similar to that described previously [23, 24]. The total CL counts were calculated by integrating the area under the curve and subtracting it from the background level. The production of CL per white blood cell (WBC) was calculated by dividing the blood CL levels by the WBC count, and expressed as CL/WBC.

### Statistical analysis

All data were expressed as Mean ± SD. All statistical analyses were performed by using GB-STAT

TABLE I. *General characteristics of type 2 diabetic patients.*

| | BL | CE | BL + CE |
|---|---|---|---|
| No. of patients | 12 | 11 | 12 |
| Male/Female | 8/4 | 9/2 | 8/4 |
| Age (years) | 63 ± 4 | 55 ± 8 | 59 ± 7 |
| BMI ($kg/m^2$) | 25.8 ± 2.4 | 23.1 ± 3.0 | 24.8 ± 2.7 |
| $HbA_{1c}$* (%) | 7.8 ± 1.9 | 7.7 ± 2.2 | 7.5 ± 1.1 |
| FG* (mmol/L) | 8.9 ± 1.3 | 9.7 ± 1.7 | 9.8 ± 1.7 |
| Duration of diabetes (years) | 14 ± 5 | 10 ± 4 | 12 ± 3 |

All values are Mean ± S.D.
Abbreviations: BL (young barley leaf extract group) ; C + E (vitamins C and E group) ; BL + C + E (young barley leaf extract plus vitamins C and E) ; FG (fasting plasma glucose) ; $HbA_{1c}$ (glycated hemoglobin) ; BMI (body mass index).
* Data assayed before the experiment, were used for selecting the patients (FG > 7.7 mmol/L & $HbA_{1c}$ > 6%) for the study.

TABLE II. *Effect of antioxidants on plasma fasting glucose, glycated hemoglobin and lipids.*

| | BL | CE | BL + CE |
|---|---|---|---|
| FG (mmol/L) | | | |
| – Week 0 | 10.1 ± 2.0[a] | 10.5 ± 1.8[a] | 10.7 ± 2.9[a] |
| – Week 4 | 10.0 ± 1.8[a] | 9.4 ± 1.0[a] | 10.3 ± 3.7[a] |
| $HbA_{1c}$ (%) | | | |
| – Week 0 | 8.0 ± 2.[a] | 7.8 ± 1.2[a] | 7.7 ± 1.8[a] |
| – Week 4 | 8.1 ± 2.[a] | 7.6 ± 1.5[a] | 7.4 ± 1.4[a] |
| TG (mmol/L) | | | |
| – Week 0 | 2.3 ± 0.7[a] | 2.4 ± 0.5[a] | 2.5 ± 0.5[a] |
| – Week 4 | 2.1 ± 0.3[a] | 1.9 ± 0.3[b] | 2.1 ± 0.5[a] |
| Cholesterol (mmol/L) | | | |
| – Week 0 | 7.0 ± 1.2[a] | 7.2 ± 0.7[a] | 7.3 ± 0.8[a] |
| – Week 4 | 6.4 ± 1.0[b] | 6.1 ± 0.9[b] | 6.4 ± 0.8[b] |
| LDL-C (mmol/L) | | | |
| – Week 0 | 4.5 ± 0.5[a] | 4.7 ± 0.6[a] | 4.8 ± 0.6[a] |
| – Week 4 | 3.9 ± 0.8[b] | 3.9 ± 0.9[b] | 3.9 ± 0.7[b] |
| HDL-C (mmol/L) | | | |
| – Week 0 | 1.4 ± 0.3[a] | 1.4 ± 0.3[a] | 1.5 ± 0.3[a] |
| – Week 4 | 1.5 ± 0.2[a] | 1.5 ± 0.2[a] | 1.6 ± 0.3[a] |

All values are Mean ± S.D.
Abbreviations: BL (young barley leaf extract group) ; CE (vitamins C and E group) ; BL+CE (young barley leaf extract plus vitamins C and E) ; FG (fasting plasma glucose) ; $HbA_{1c}$ (glycated hemoglobin) ; TG (triacylglycerol) ; LDL (low density lipoprotein) ; HDL (high density lipoprotein).
[a] Data within the same row and the same column of the same parameter not sharing a common superscript letter were significantly different, $P < 0.05$.
[b] Data within the same row and the same column of the same parameter not sharing a common superscript letter were significantly different, $P < 0.05$.

(Version 5.0; Dynamic Microsystems, Inc., USA). Comparisons were done by one-way ANOVA among different groups. Turkey's post hoc test was used to analyze significant effects. Paired student-*t*-tests were used to determine the differences between values of 0 week and 4 weeks, B-LDL and Sd-LDL within the groups. In order to control for the confounding effects of baseline values, we derive percentage of change (value of week 4 - value of week 0/value of week 0). Then a two-factor randomized block design was applied to evaluate the effects of treatments on different LDL subfractions within and among the groups. The BL, CE and BL + CE groups were treated as independent. Values of B-LDL and Sd-LDL were treated as block in the two-factor randomized block design. A *P* value of 0.05 was taken as the threshold for statistical significance.

## ■ RESULTS

### Subject characteristics

Thirty-five of thirty-six participants completed the trial. One subject in the vitamins C and E group removed herself from the study due to personal reasons. The characteristics of the participants are displayed in *Table I*. There were no significant differ-

TABLE III. *Effect of antioxidants on oxidation of LDL subfractions.*

| | B-LDL | | Sd-LDL | |
|---|---|---|---|---|
| | **Week 0** | **Week 4** | **Week 0** | **Week 4** |
| LDL-vitamin E | | | | |
| – BL | 2.7 ± 0.8[a] | 4.6 ± 1.9[b] | 2.7 ± 1.1[a] | 4.3 ± 2.2[b] |
| – CE | 2.3 ± 0.8[a] | 4.4 ± 1.6[b] | 2.1 ± 1.3[a] | 4.9 ± 3.2[b] |
| – BL + CE | 2.4 ± 1.5[a] | 6.5 ± 3.4[c] | 2.0 ± 0.9[a] | 6.5 ± 3.9[c] |
| Lag time (minutes) | | | | |
| – BL | 98 ± 21[b] | 144 ± 31[c] | 80 ± 20[a] | 114 ± 40[bc] |
| – CE | 94 ± 15[b] | 134 ± 29[c] | 68 ± 17[a] | 106 ± 34[b] |
| – BL + CE | 95 ± 24[b] | 171 ± 43[d] | 67 ± 18[a] | 176 ± 66[d] |
| $\Delta A_{234}$ | | | | |
| – BL | 0.36 ± 0.11[a] | 0.32 ± 0.10[a] | 0.50 ± 0.11[a] | 0.40 ± 0.14[a] |
| – CE | 0.38 ± 0.10[a] | 0.24 ± 0.18[a] | 0.40 ± 0.20[a] | 0.26 ± 0.11[a] |
| – BL + CE | 0.37 ± 0.10[a] | 0.29 ± 0.12[a] | 0.39 ± 0.15[a] | 0.25 ± 0.09[a] |
| Propagation rate (ΔOD/min) | | | | |
| – BL | 0.006 ± 0.002[a] | 0.005 ± 0.002[a] | 0.010 ± 0.006[a] | 0.009 ± 0.003[a] |
| – CE | 0.006 ± 0.003[a] | 0.005 ± 0.002[a] | 0.010 ± 0.008[a] | 0.005 ± 0.002[a] |
| – BL + CE | 0.005 ± 0.003[a] | 0.006 ± 0.003[a] | 0.008 ± 0.008[a] | 0.006 ± 0.002[a] |

All values are mean ± S.D.
LDL-vitamin E values are in micrograms of α-tocopherol per milligram of LDL protein. For B-LDL, d = 1.022-1.034 g/ml ; and Sd-LDL, d = 1.040-1.054 g/ml. Abbreviations: BL (young barley leaf extract group) ; CE (vitamins C and E group) ; BL + CE (young barley leaf extract plus vitamins C and E) ; LDL (low density lipoprotein)
[a] Data within the same row and the same column of the same parameter not sharing a common superscript letter were significantly different at $P < 0.05$.
[b] Data within the same row and the same column of the same parameter not sharing a common superscript letter were significantly different at $P < 0.05$.
[c] Data within the same row and the same column of the same parameter not sharing a common superscript letter were significantly different at $P < 0.05$.
[d] Data within the same row and the same column of the same parameter not sharing a common superscript letter were significantly different at $P < 0.05$.

ences among the three groups in terms of age, body mass index, $HbA_{1c}$, fasting plasma glucose or duration of diabetes.

## Plasma glucose and lipids

The values for plasma glucose, lipids and lipoproteins are shown in *Table II*. There were no significant changes in fasting glucose, $HbA_{1c}$ and TG after antioxidants supplementation, except a reduction of TG in CE group. Participants in all groups had significantly decreased levels of plasma total cholesterol (TC) and LDL-C. However, no further decreases of plasma TC or LDL-C concentrations occurred in the group that received BL plus antioxidative vitamins C and E, comparing with the BL group.

## Effects on LDL susceptibility to oxidation

The data of LDL susceptibility to oxidation are shown in *Table III*. The vitamin E contents in B-LDL and Sd-LDL were increased significantly following supplementation with each antioxidant treatment. It

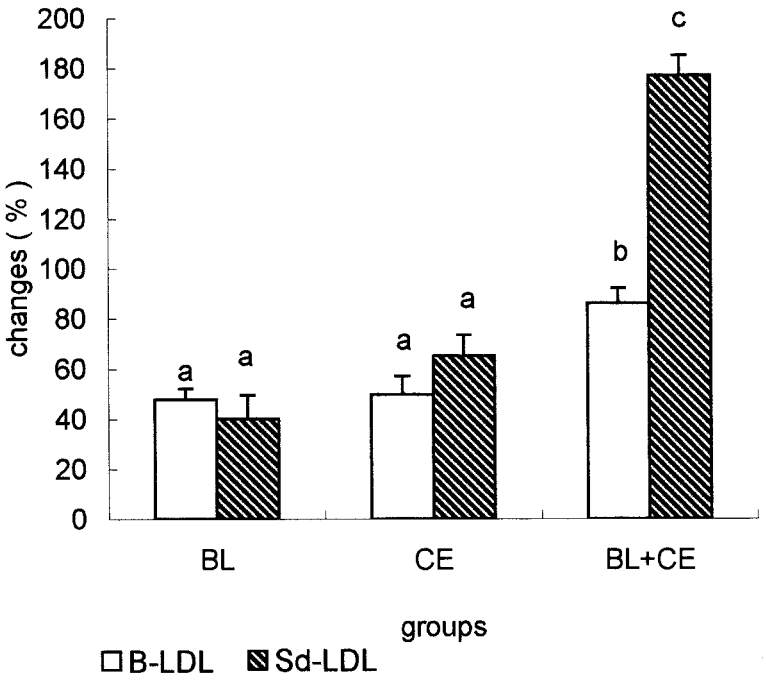


FIG. 1. *The percent increase of lag time after 4 weeks antioxidants supplementation. Abbreviations: BL (young barley leaf extract group); CE (vitamins C and E group); BL + CE (young barley leaf extract plus vitamins C and E).*
*[a-c] Means with different letters are significantly different at P < 0.05 among the different groups.*

was noticed that the vitamin E contents in both B-LDL and Sd-LDL were higher in the BL + CE group than either the BL or CE group. The lag times of Sd-LDL of week 0 were shorter than those of B-LDL. Lag times of B-LDL and Sd-LDL increased significantly at week 4 as compared to week 0 in all three groups. Moreover, the lag time of BL + CE group was longer than that of BL or CE group. The propagation rate and $\Delta A_{234}$ remained unchanged after supplementation. The percent increase of lag phase in the BL group was almost the same as that of the vitamins C and E group *(Fig. 1)*. The percent increases of lag phase in the group receiving BL plus vitamins C and E were significantly higher (86 ± 9.3% in B-LDL, 177.1 ± 8.1% in Sd-LDL, respectively) than those of the group receiving BL (47.8 ± 4.4% in B-LDL, 39.9 ± 9.7% in Sd-LDL, respectively), or vitamins C and E (49.8 ± 7.3% in B-LDL, 65 ± 8.5% in Sd-LDL, respectively) alone. The antioxidative effect of BL + CE group was the greatest for Sd-LDL with 4 folds (177.1 ± 8.1%) increases in percentage of lag time compared to BL alone (39.9 ± 9.7%) *(Fig. 1)*.

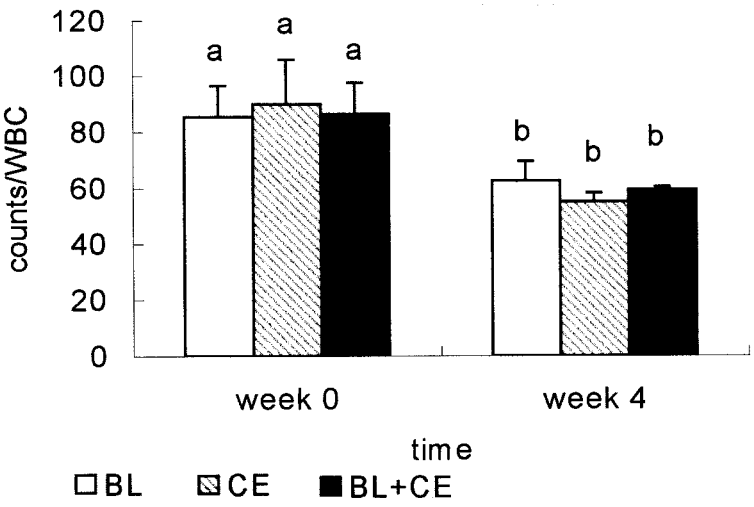


FIG. 2. *The effects of antioxidants on the whole blood lucigenin-CL count.*
*Abbreviations: CL (chemiluminescence); BL (young barley leaf extract group); CE (vitamins C and E group); BL + CE (young barley leaf extract plus vitamins C and E).*
*[a-b] Means with different letters are significantly different at P < 0.05 among the different groups.*

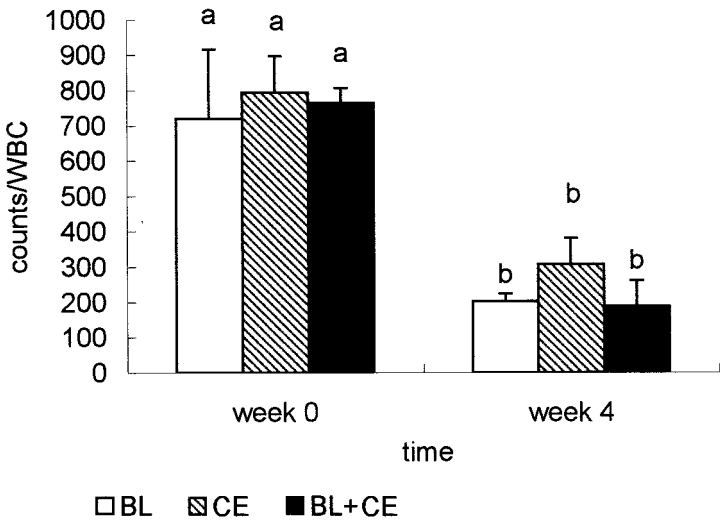


FIG. 3. *The effects of antioxidants on whole blood luminol-CL count.*
*Abbreviations: CL (chemiluminescence); BL (young barley leaf extract group); CE (vitamins C and E group); BL + CE (young barley leaf extract plus vitamins C and E).*
*[a-b] Means with different letters are significantly different at P < 0.05 among the different groups.*

### Effect on free radical scavenging activities

The lucigenin-CL levels of the whole blood significantly decreased 29.4 ± 8.7% or 40.8 ± 24.0% after 4 weeks of BL or vitamins C and E supplementation. For the group of BL + CE, the corresponding decrease was 38.6 ± 7.1% ($P < 0.05$) *(Fig. 2)*. This result suggests that supplementation with BL or vitamins C and E reduced the production of superoxide radicals. A combination of BL(vitamins C and E, however, could not enhance this reduction.

The luminol-CL levels of the whole blood also significantly decreased 66.6 ± 26.6%, 53.4 ± 20.1% and 65.4 ± 11.4% respectively after 4 weeks of BL, CE or BL + CE supplementation *(Fig. 3)*. These results indicate that the production of oxygen free radicals by peripheral blood leukocytes was significantly reduced after supplementation with antioxidants. However, it was observed that the combination of these two types of antioxidants did not increasingly inhibit the production of oxygen free radicals.

## ■ DISCUSSION

This study found that supplementation with either 15 g/d BL or vitamins C (200 mg/d) and E (200 mg/d) for 4 weeks significantly decreased levels of plasma TC and LDL-C. A similar result reported by Khajehdehi [25] demonstrated that vitamin C (200 mg) and vitamin E (200 mg) reduced plasma TC, LDL-C as well as the ratios of LDL-C/HDL-C levels in patients undergoing hemodialysis. Although the efficacy of vitamins C and E in lowering plasma lipids remains controversial, our results support the notion that these antioxidants possess hypocholesterolemic properties *(Table II)*. In addition, there were no further decreases in plasma TC levels in the group that received BL in combination with vitamins C and E.

Our study also evaluated the effects of ingestion of BL, which has never been studied *in vivo*, alone or with other antioxidants on susceptibilities of LDL subfractions to oxidation in type 2 diabetes. Evaluation of these interventions in type 2 DM subjects is particularly relevant because their LDL is frequently more enriched in Sd-LDL particles [1], more susceptible to oxidation [2], and elevates oxygen free radical formation in blood [6, 26].

In biological systems, the source of oxygen free radicals may be superoxide radicals, hydrogen peroxide, or hydroxyl radicals [27]. Luminol-CL has been used as a sensitive indicator of oxygen free radical production [23]. Lucigenin-CL reacts more specifically with superoxide anions that are generated by NADP (H) oxidase in leukocytes [23, 24]. Our results showed that supplementation with BL or vitamins C and E reduced the levels of oxygen free radicals in blood. This indicates that BL acts as an oxygen radical scavenger. However, no further decrease was observed in the BL + CE group. Its ability to scavenge free radicals may be derived from the polyphenolic structure of BL.

Previous studies have shown that supplementation of vitamin E in type 2 diabetes prolongs the lag phase of B-LDL oxidation more effectively than Sd-LDL. Thus, Sd-LDL may provide less protection against oxidation from vitamin E than B-LDL. Combinations of various antioxidants may be important in providing LDL with protection from oxidation [12]. Our results demonstrated that the combination of BL and vitamins C + E may prolong the lag time by 86 ± 9.3% for B-LDL oxidation, and by 177.1 ± 8.1% for Sd-LDL. Therefore, the benefit of BL + CE was greatest for

Sd-LDL, with a 4 fold increase in lag time compared to BL alone *(Fig. 1)*.

In conclusion, supplementation of BL reduced the plasma levels of TC and LDL-C. The percent increase of lag times in the BL + CE group was significantly higher than those in the BL or CE group. The greatest antioxidative benefit of adding BL with vitamins C and E was for Sd-LDL. It was also observed that BL decreased lucigenin-CL and luminol-CL levels in blood. These results suggest that the production of oxygen free radicals is effectively inhibited by BL. Therefore, supplementation of BL in combination with antioxidative vitamins can reduce some major risk factors of atherosclerosis. This may protect type 2 diabetic patients from vascular diseases.

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