JMF-2010-1195-ver9-Chen_1P
Type: full-communication

JOURNAL OF MEDICINAL FOOD
J Med Food 14 (0) 2011, 1–6

DOI: 10.1089/jmf.2010.1195

FULL COMMUNICATION

Efficacy of Epigallocatechin-3-Gallate and Amla (*Emblica officinalis*) Extract for the Treatment of Uremic Diabetic Patients

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ABSTRACT Uremic patients with diabetes suffer from high levels of oxidative stress due to regular hemodialysis therapy (neutrophil activation induced by hemo-incompatibility between the hemodialyser and blood) and complications associated with diabetes. Several plasma biomarkers were screened in 13 uremic diabetic patients after receiving the mixture of (–)-epigallocatechin gallate (EGCG), a major component of green tea extract, and Amla extract (AE), from *Emblica officinalis*, the Indian gooseberry, for 3 months. We found that oral administration of a 1:1 mixture of EGCG and AE for 3 months significantly improved antioxidant defense as well as diabetic and atherogenic indices in uremic patients with diabetes. Furthermore, no significant changes in hepatic function, renal function, or inflammatory responses were observed. These results suggest that a 1:1 combination of EGCG and AE is a safe and effective treatment for uremic patients with diabetes.

KEY WORDS: • *Amla* • *atherosclerosis* • *diabetes* • Emblica officinalis • *(–)-epigallocatechin gallate* • *oxidative stress* • *uremia*

INTRODUCTION

HEMODIALYSIS IS ONE of the therapeutic strategies used for patients with end-stage renal failure (uremic patients). Although the hemodialysis process assists uremic patients in the removal of low-molecular-weight metabolic waste materials (uremic solutes), it is capable of activating neutrophils because of the hemo-incompatibility between the hemodialyser (artificial kidney) and blood.[1] The activated neutrophils release large amounts of reactive oxygen species into the blood, which results in increased oxidative stress. This is called a neutrophil burst.[2]

In addition to hemodialysis, several factors, including infection,[3] overdose of iron supplements,[4,5] and complications,[6] increase the level of oxidative stress in uremic patients. Among these factors, complications resulting from uremia are the most frequent cause of oxidative stress because most of these complications are not easily cured. Furthermore, reduced renal function may increase accumulation of reactive aldehyde and oxidized thiol or other metabolic wastes that contribute to increased oxidative stress and/or decreased levels of reduced glutathione.

This study focused on uremic patients with diabetes. Regular hemodialysis and diabetic complications lead to high levels of oxidative stress in these patients.[7,8] Supplementing antioxidants might be a convenient and economic strategy for reducing oxidative stress in uremic patients with diabetes. *In vitro* and *in vivo* studies of herbal extracts for treating uremic diabetic patients have provided evidence that (–)-epigallocatechin gallate (EGCG), the major component of green tea extract, is a powerful antioxidant and reactive oxygen species scavenger.[9,10] Amla, an extract of *Emblica officinalis* (the Indian gooseberry), was shown to decrease the production of advanced glycosylated end-products (AGEs) and 5-hydroxymethylfurfural (glycosylated protein) *in vivo*.[11,12] This study was designed to investigate the potential for use of a combination of EGCG and Amla extract (AE) in the treatment of uremic patients with diabetes.

First, the optimal ratio of EGCG/AE was investigated by several *in vitro* experiments, including the antioxidant power and inhibition of AGE production with different EGCG/AE ratios. Once the optimal ratio of EGCG/AE combination was confirmed, tests were conducted in uremc diabetic patients before and after receiving the optimal ratio of EGCG/AE for 3 months, including plasma antioxidant power, oxidative

Manuscript received 1 June 2010. Revision accepted 23 March 2011.

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stress markers, diabetic indices, atherogenic markers, inflammatory markers, hepatic function, and renal function.

MATERIALS AND METHODS

Reagents

All reagents used in this study were from Sigma-Aldrich Inc. (St. Louis, MO, USA). Standards for investigating clinical biomarkers were from Randox Laboratories (Crumlin, County Antrim, UK).

Plant extract

The extracts from green tea (EGCG) (batch number 911011) and from *E. officinalis* (AE) (batch number 608291) were obtained from Numen Biotech Co., Ltd. (Taipei, Taiwan). The purity of EGCG was greater than 90% (>90% of EGCG and <0.1% of caffeine content). On the other hand, the most abundant active compounds existing in AE are emblicanin A and emblicanin B.[13] According to the information provided from Numen Biotech Co., Ltd., AE contained 30% of polyphenols (colorimetric method) and 2% of vitamin C (by high-performance liquid chromatography analysis).

Participants

The baseline characteristic for the 13 uremic patients with diabetes and 15 healthy volunteers enrolled in this study are given in Table 1. The EGCG/AE tablet was administered orally (one tablet per time, three times per day) to uremic diabetic patients for 3 months. Each tablet contained 100 mg of EGCG, 100 mg of AE, and 50 mg of excipient (starch). The subjects were instructed to consume no EGCG, AE-related foods, or additional drugs and to make no dietary changes during the experimental period. The data for the healthy volunteers served as normal ranges. Informed consent was obtained from patients for oral administration of herbal extracts and plasma utilization.

Ferric reducing/antioxidant power assay

The ferric reducing/antioxidant power (FRAP) assay for this experiment was modified from the procedure of Othman *et al*.[14] The FRAP reagent was prepared by mixing acetate buffer (246.1 mg of sodium acetate in 10 mL of 10% acetic acid), Tris(pyridyl)-*s*-triazine solution [31.23 mg of Tris(-pyridyl)-*s*-triazine and 0.044 mL of 37% HCl in 10 mL of distilled water], and $FeCl_3$ solution (54.06 mg of $FeCl_3 \cdot 6H_2O$ in 10 mL of distilled water) in a ratio of 10:1:1 (by volume). The FRAP reagent (0.5 mL), distilled water (0.05 mL), and plasma samples (with or without plant extracts) were placed into 1.5-mL vials and kept at room temperature. After 4 minutes, the absorbance at 593 nm was measured for all vials. During the experiment, distilled water and $FeSO_4$ functioned as blank and standard, respectively. The FRAP data are expressed as $FeSO_4$ equivalents.

Inhibition of AGEs formation

This protocol was modified from that of Nakagawa *et al*.[15] In brief, 1.2 mL of solution containing bovine serum albumin (10 mg of bovine serum albumin dissolved in 1 mL of 50 m*M* phosphate buffer [pH 7.4], 0.02% sodium azide, 25 m*M* glucose, and 25 m*M* fructose) and different ratios of EGCG/AE (100 μg/mL) were placed into vials and incubated at 37°C for 2 weeks. After incubation, the fluorescent intensity for all vials was determined with an excitation wavelength of 350 nm and an emission wavelength of 450 nm. The reaction mixture was also investigated at zero-time, and the fluorescent data for this time point served as a control. The AGEs inhibitory effect for different ratios of EGCG/AE combinations is expressed as a percentage of the control value.

Plasma nitrogen oxides

The level of plasma nitrogen oxides (NOx) was measured using the Griess reagent.[16] In brief, plasma samples (with or without plant extracts) and Griess reagent were mixed at a 1:1 ratio, kept at room temperature for 5 minutes, and then centrifuged at 13,500 *g* for 2 minutes. The supernatant was collected, and the absorbance at 540 nm was measured. Deionized water served as a blank. All data are expressed as sodium nitrite ($NaNO_2$) equivalents.

Plasma thiobarbituric acid–reactive substances

The process was modified from that of Ohkawa *et al*.[17] In brief, 0.1 mL of plasma sample, 0.1 mL of normal saline, 0.2 mL of 20% trichloroacetic acid, and 0.05 mL of thiobarbituric acid reagent (20 mg of thiobarbituric acid in 6 mL of 50% acetic acid) were placed into 1.5-mL vials and incubated in a water bath at 85°C for 1.5 hours. After incubation, 0.6 mL of *n*-butanol was added, and the mixture was subjected to low-speed centrifugation at room temperature for 10 minutes. The supernatant was collected, and the absorbance at 520 nm was measured. Normal saline served as a blank. All data are expressed as malondialdehyde equivalents.

Table 1. Plasma Diabetic Markers for Uremic Patients After Receiving 1:1 (–)-Epigallocatechin Gallate /Amla Extract for 3 Months

Participants	*Gender*	*Age (years)*	*Hemodialysis duration (months)*	*Blood glucose (mg/dL)*
Normal ($n=15$)	F, 9; M, 6	49±17	—	100.9±13.8
Uremic-DM ($n=13$)	F, 9; M, 4	66±16	86±65	171.9±73.3

Normal participants were healthy volunteers. Uremic-DM participants were uremic patients with diabetes mellitus.
F, female; M, male.

Plasma clinical markers

Clinical markers such as hepatic function, renal function, atherogenic indices, and biomarkers for diabetes and inflammation were screened with a clinical analyzer (IMMULITE, DPC Cirrus Inc., Los Angeles, CA, USA).

Data analysis

All data are expressed as mean ± SD values. Student's *t* test was used for data analysis, and statistical significance was set at $P < .05$.

RESULTS

Optimal ratio of EGCG/AE

F1 ▶ Figure 1A shows the FRAP values before and after adding different ratios of EGCG/AE to plasma. Plasma FRAP is approximately proportional to plasma antioxidant power. We found that the results of plasma FRAP before and after adding EGCG and AE in different ratios into plasma were as follows: plasma only, 111 ± 1 *μM*; plasma + 1:9 EGCG/AE, 118 ± 1 *μM*; plasma + 2:8 EGCG/AE, 158 ± 1 *μM*; plasma + 3:7 EGCG/AE, 138 ± 3 *μM*; plasma + 4:6 EGCG/AE, 138 ± 2 *μM*; plasma + 5:5 EGCG/AE, 178 ± 1 *μM*; plasma + 6:4 EGCG/AE, 158 ± 1 *μM*; plasma + 7:3 EGCG/AE, 165 ± 3 *μM*; plasma + 8:2 EGCG/AE, 171 ± 1 *μM*; and plasma + 9:1 EGCG/AE, 178 ± 2 *μM*. We found that plasma + 5:5 EGCG/AE had the highest plasma FRAP among these ratios (plasma only vs. 5:5 ratio, $P < .05$).

Figure 1B shows the inhibitory effect of AGEs production after adding EGCG/AE in different ratios to a solution of bovine serum albumin. The inhibitory effect of AGEs was as follows: 1:9, 27.61 ± 0.77%; 2:8, 26.87 ± 1.26%; 3:7, 26.15 ± 1.01%; 4:6, 38.60 ± 1.25%; 5:5, 40.31 ± 2.94%; 6:4, 42.83 ± 1.59%; 7:3, 39.82 ± 2.56%; 8:2, 40.55 ± 2.56%; and 9:1, 38.52 ± 1.83%, respectively. Figure 1B can be divided into two groups based on AGEs inhibitory percentage of EGCG/AE combinations: high AGEs inhibitory group (including 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1) and low AGEs inhibitory group (including 1:9, 2:8, and 3:7). We found that no significant difference was observed when 5:5 was compared with 4:6, 6:4, 7:3, 8:2, or 9:1. This means that 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1 statistically show similar AEGs inhibitory ability. On the other hand, a significant difference ($P < .01$) was observed when 5:5 was compared with 1:9, 2:8, or 3:7. Based on the above data, we suggest that 5:5 is the statistically optimal ratio for AGEs inhibition.

Figure 1C shows the level of plasma NOx (an oxidative stress marker) before and after adding different ratios of EGCG/AE into the plasma. The results were as follows: plasma only, 500 ± 3 ng/mL; 1:9, 513 ± 3 ng/mL; 2:8, 538 ± 3 ng/mL; 3:7, 525 ± 2 ng/mL; 4:6, 500 ± 4 ng/mL; 5:5, 488 ± 2 ng/mL; 6:4, 575 ± 5 ng/mL; 7:3, 525 ± 5 ng/mL; 8:2, 538 ± 3 ng/mL; and 9:1, 500 ± 4 ng/mL, respectively. The 5:5 ratio of EGCG/AE showed the best potential for suppression of plasma NOx formation (plasma only vs. 5:5 ratio, $P < .001$).

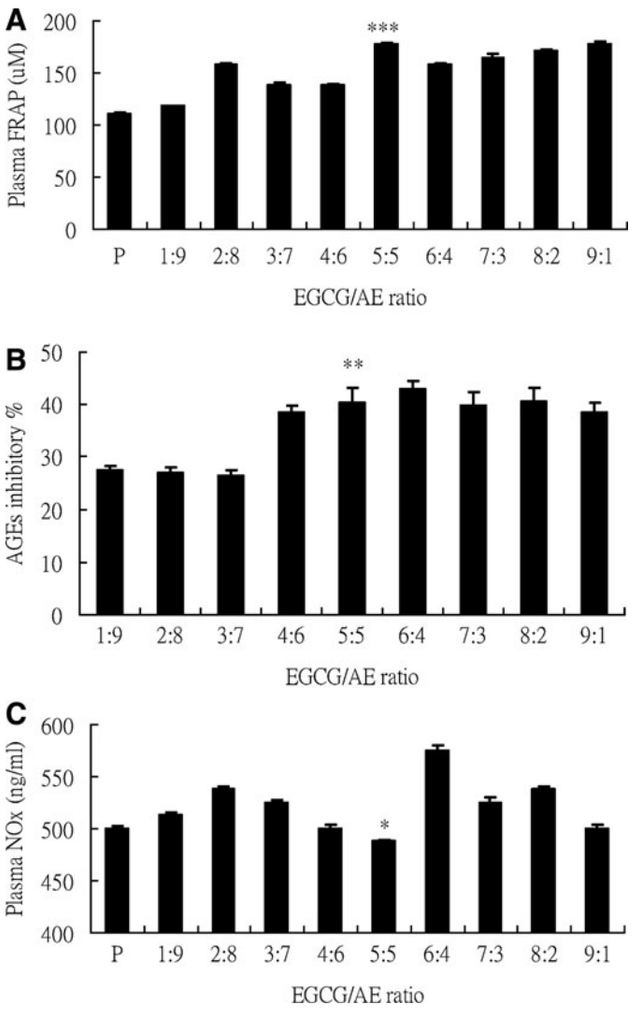

FIG. 1. Determination of the optimal (–)-epigallocatechin gallate (EGCG):Amla extract (AE) ratio based on (**A**) plasma ferric reducing/antioxidant power (FRAP) assay, (**B**) advanced glycosylated end products (AGEs) inhibitory percentage, and (**C**) plasma nitrogen oxides (NOx) assay. Plasma FRAP and plasma NOx were determined before (plasma-only [P]) and after adding EGCG/AE in different ratios to plasma. AGEs inhibitory percentage was determined after adding EGCG/AE in different ratios to a solution containing bovine serum albumin, glucose, and fructose. *$P < .05$, ***$P < .001$ compared with P; **$P < .01$ compared with 1:9. Data are mean ± SD values ($n = 3$).

As mentioned above, 5:5 (or 1:1) EGCG/AE exhibited excellent activity in increasing plasma FRAP (antioxidant), inhibiting AGEs (glycosylated products) production, and suppressing plasma NOx (oxidative stress) formation. On the basis of these results, 1:1 EGCG/AE was the optimal combination for the following experiments.

Biochemical tests in uremic diabetic patients at zero-time and after receiving 1:1 EGCG/AE for 3 months

Plasma antioxidant/oxidative status. Figure 2A shows ◀ F2 the plasma FRAP determined in the healthy volunteers (Normal, served as the normal range) and uremic diabetic

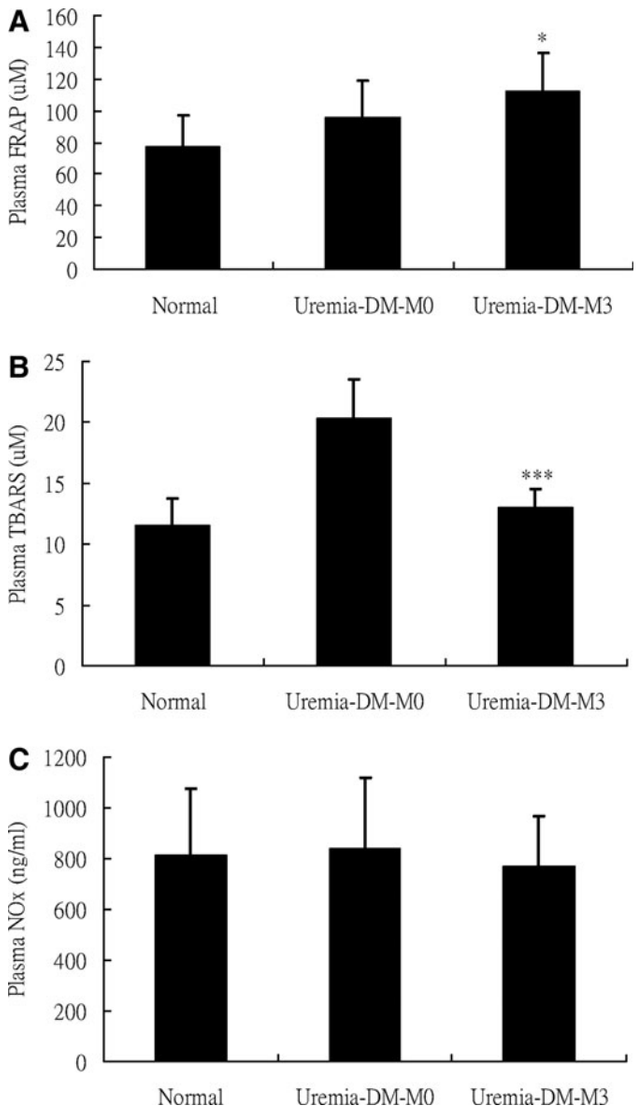

FIG. 2. Plasma antioxidant status and oxidative markers for uremic patients after receiving 1:1 EGCG/AE for 3 months: healthy volunteers (Normal) ($n = 15$) and uremic diabetic patients ($n = 13$) at zero-time (Uremic-DM-M0) and after receiving 1:1 EGCG/AE for 3 months (Uremic-DM-M3): (**A**) plasma FRAP (antioxidant index), (**B**) plasma thiobarbituric acid–reactive substances (TBARS) (oxidative marker), and (**C**) plasma NOx (oxidative marker). Data are mean ± SD values. Significant differences (compared with Uremic-DM-M0) are indicated: $*P < .05$, $***P < .001$.

patients at zero-time (Uremic-DM-M0) and after receiving 1:1 EGCG/AE for 3 months (Uremic-DM-M3). The results were as follows: Normal, 76.80 ± 22.34 μM; Uremic-DM-M0, 95.38 ± 23.88 μM; and Uremic-DM-M3, 112.15 ± 24.07 μM. The values for Uremic-DM-M0 and Uremic-DM-M3 were significantly different (Uremic-DM-M0 < Uremic-DM-M3, $P < .05$).

Figure 2B shows results for plasma thiobarbituric acid–reactive substances, a plasma oxidative stress marker, for healthy volunteers and patients. The data were as follows: Normal, 11.49 ± 2.29 μM; Uremic-DM-M0, 20.31 ± 3.2 μM; and Uremic-DM-M3, 12.92 ± 1.66 μM. The values for Uremic-DM-M0 and Uremic-DM-M3 were significantly different (Uremic-DM-M0 > Uremic-DM-M3, $P < .001$).

Figure 2C shows the results for plasma NOx (one of the plasma oxidative stress markers) for healthy volunteers and patients. The data were as follows: Normal, 813 ± 263 ng/mL; Uremic-DM-M0, 837 ± 283 ng/mL; and Uremic-DM-M3, 66 ± 201 ng/mL. The difference between the Uremic-DM-M0 and Uremic-DM-M3 values was not significant.

Diabetic markers. Figure 3 summarizes the levels of plasma diabetic markers in uremic diabetic patients before and after receiving 1:1 EGCG/AE for 3 months. In Figure 3A, the levels of plasma glucose were as follows: Normal, 100.93 ± 13.77 mg/dL; Uremic-DM-M0, 171.92 ± 13.77 mg/dL; and Uremic-DM-M3, 134.62 ± 25.43 mg/dL. The values for Uremic-DM-M0 and Uremic-DM-M3 were significantly different (Uremic-DM-M0 > Uremic-DM-M3, $P < .05$). ◀F3

Figure 3B shows the results for glycosylated hemoglobin for healthy volunteers and patients. The data were as follows: Normal, 4.5 ± 0.2%; Uremic-DM-M0, 7.54 ± 0.79%; and Uremic-DM-M3, 7.50 ± 0.96%. The values for Uremic-DM-M0 and Uremic-DM-M3 were not significantly different.

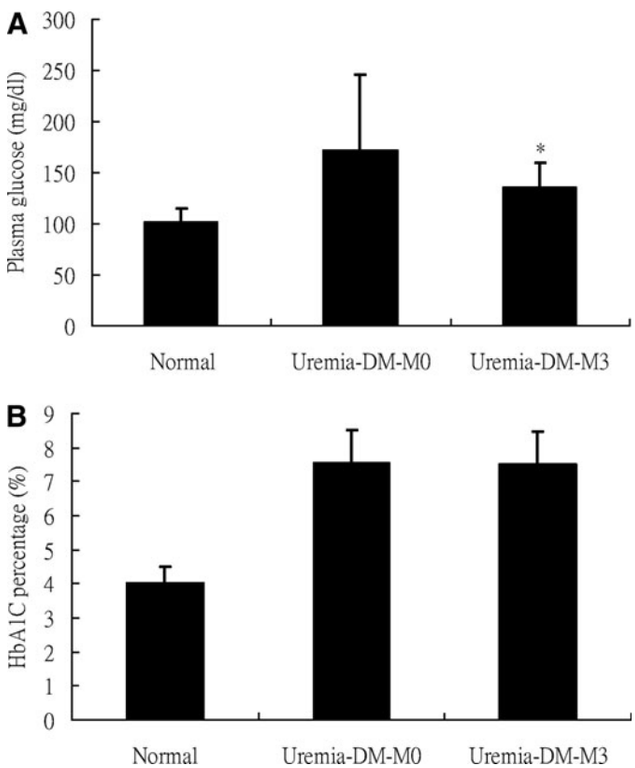

FIG. 3. Plasma diabetic markers for uremic patients after receiving 1:1 EGCG/AE for 3 months: Normal ($n = 15$) and Uremic-DM-M0 and Uremic-DM-M3 ($n = 13$): (**A**) plasma glucose and (**B**) plasma glycosylated hemoglobin (HbA1c). Data are mean ± SD values. Significant differences (compared with Uremic-DM-M0) are indicated: $*P < .05$.

Atherogenic markers. Several atherogenic markers were examined in uremic diabetic patients before and after receiving 1:1 EGCG/AE for 3 months. Table 2 shows that Uremic-DM-M0 and Uremic-DM-M3 were significantly different for the markers of high-density lipoprotein (HDL) (Uremic-DM-M0 < Uremic-DM-M3, $P < .05$) and the low-density lipoprotein (LDL)/HDL ratio (Uremic-DM-M0 > Uremic-DM-M3, $P < .05$). The decrease in the LDL/HDL ratio (Uremic-DM-M0 > Uremic-DM-M3) is likely to be due to the increased HDL in Uremic-DM-M3.

Hepatic and renal functions. Table 3 summarizes the results for hepatic and renal functions in patients before and after receiving 1:1 EGCG/AE for 3 months. Differences between Uremic-DM-M0 and Uremic-DM-M3 for all markers were not statistically significant.

Inflammatory marker. Figure 4 shows that the plasma levels of the inflammatory marker C-reactive protein for Normal (0.07 ± 0.08 mg/dL), Uremic-DM-M0 (0.62 ± 0.88 mg/dL), and Uremic-DM-M3 (0.48 ± 0.41 mg/dL). The difference between Uremic-DM-M0 and M3 was statistically insignificant.

Table 3. Hepatic and Renal Functions in Uremic Diabetic Patients After Receiving 1:1 (–)-Epigallocatechin Gallate /Amla Extract for 3 Months

Clinical marker	*Normal*	*Uremic-DM-M0*	*Uremic-DM-M3*
ALB	4.05 ± 0.39	4.02 ± 0.29	3.94 ± 0.25
GLO	2.94 ± 0.43	3.61 ± 0.51	3.54 ± 0.53
ALP	60.27 ± 36.74	368.38 ± 155.59	428.62 ± 174.72
AST	22.01 ± 6.23	27.54 ± 13.88	33.08 ± 20.53
GPT	30.07 ± 13.85	21.77 ± 21.71	31.31 ± 25.24
(-GTP	19.41 ± 9.81	54.31 ± 61.22	44.54 ± 42.21
BUN	17.13 ± 7.15	57.08 ± 12.49	63.38 ± 14.12
Cr	0.99 ± 0.25	8.59 ± 1.87	8.38 ± 1.83
UA	5.69 ± 1.37	7.24 ± 1.44	7.98 ± 2.12
IP	3.49 ± 0.63	4.22 ± 1.75	4.64 ± 1.77

Data are mean ± SD values.

ALB, albumin (g/dL); ALP, alkaline phosphatase (U/L); ALT, alanine transaminase (U/L); AST, aspartate transaminase (U/L); BUN, blood urea nitrogen (mg/dL); Cr, creatinine (mg/dL); GLO, globulins (mg/dL); γ-GTP, γ-glutamyl transpeptidase (U/L); IP, inorganic phosphorus (mg/dL); UA, uric acid (mg/dL).

DISCUSSION

Uremic patients with diabetes suffer from high levels of oxidative stress due to regular hemodialysis therapy (neutrophil burst) and diabetic complications. Therefore, the use of herbal extracts to reduce oxidative stress and diabetes-induced pathological reactions might be a convenient, economic, and alternative therapeutic strategy for uremic patients with diabetes. From a review of several studies, we found that EGCG exhibits potent antioxidant power for reducing oxidative stress. On the other hand, AE exhibits potential in decreasing the formation of glycosylated proteins. Therefore we hypothesized that a combination of EGCG and AE may have potential for the treatment of uremic patients with diabetes. Several *in vitro* experiments were first conducted to determine the optimal ratio of EGCG/AE, for antioxidant power and AGEs inhibition. That is because uremic diabetic patients suffer from high levels of oxidative stress, and we expect that supplementation of the optimal ratio of EGCG/AE can reduce oxidative stress and decrease the risk of complications associated with it. On the other hand, uremic diabetic patients also suffer from high levels of damage induced by glucose. Therefore we investigated the AGEs inhibitory ability of different ratios of EGCG/AE. We expect that supplementation of an optimal ratio of EGCG/AE combination can reduce the damage induced by glycosylation.

Figure 1 shows that a 1:1 mixture of EGCG and AE was the optimal ratio based on investigation of antioxidant power (Fig. 1A, FRAP assay), AGEs inhibitory effect (Fig. 1B), and suppression of oxidative stress markers (Fig. 1C, plasma NOx). As a result, further investigations were done with uremic diabetic patients before and after receiving 1:1 EGCG/AE for 3 months. Thirteen uremic diabetic patients were selected based on examinations of their renal function and plasma glucose, and 15 healthy volunteers were selected as normal references. The healthy subjects were significantly younger than the uremic diabetic subjects. Earlier studies used dosages of 800 mg of EGCG/day[18] and 450 mg of AE/day for a human study.[19] We used dosages of 300 mg of EGCG and 300 mg of AE/day in this study. The plasma antioxidant/oxidative status was investigated in uremic patients with diabetes after they received 1:1 EGCG/AE for 3 months. We found that the plasma FRAP value was significantly improved and the plasma thiobarbituric acid–reactive substances (oxidative marker) level was significantly decreased. These results indicate that 1:1 EGCG/AE

Table 2. Determination of Plasma Atherogenic Indices in Uremic Diabetic Patients After Receiving 1:1 (–)-Epigallocatechin Gallate /Amla Extract for 3 Months

Atherogenic indices	*T-CHO*	*TG*	*HDL*	*LDL*	*LDL/HDL ratio*	*HCY*
Normal	195.07 ± 39.18	180.69 ± 113.31	58.67 ± 9.15	106.67 ± 24.56	1.85 ± 0.46	6.94 ± 3.06
Uremic-DM-M0	176.77 ± 25.78	207.69 ± 48.73	30.85 ± 6.04	104.38 ± 22.57	3.46 ± 0.85	22.97 ± 7.71
Uremic-DM-M3	183.77 ± 33.71	200.23 ± 59.96	36.77 ± 8.03*	105.54 ± 27.51	2.91 ± 0.67*	21.95 ± 4.56

Data are mean ± SD values.

Significant differences (compared with Uremic-DM-M0) are indicated: *$P < .05$.

HCY, homocysteine (mmol/L); HDL, high-density lipoprotein (mg/dL); LDL, low-density lipoprotein (mg/dL); T-CHO, total cholesterol (mg/dL); TG, triglycerides (mg/dL).

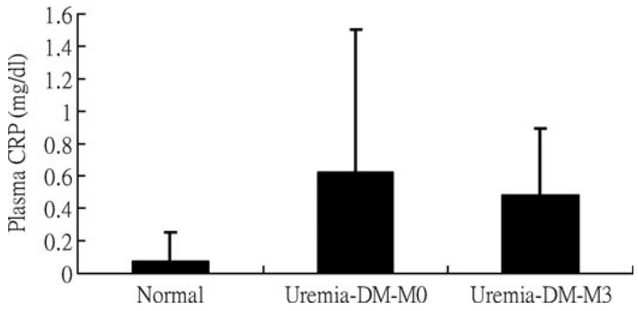

FIG. 4. Inflammatory indicator of C-reactive protein (CRP) in uremic patients after receiving 1:1 EGCG/AE for 3 months: Normal ($n = 15$) and Uremic-DM-M0 and Uremic-DM-M3 ($n = 13$). Data are mean ± SD values.

was capable of enhancing antioxidant defense in uremic patients with diabetes. An earlier study showed that polyphenol-rich extracts led to the increase of plasma antioxidant defense due to the elevated level of plasma polyphenols.[20] In this study, the polyphenol contents of EGCG and AE were 90% and 30%, respectively. Both extracts contained a high level of polyphenols. Therefore, we suggest that the increase of antioxidant power in uremic diabetic patients receiving 1:1 EGCG/AE was due to the elevated level of plasma polyphenols after ingestion of these polyphenol-rich extracts.

In addition to antioxidant defense, several clinical markers were investigated in the uremic diabetic patients in this study. After the 3-month trial, the levels of plasma glucose, plasma HDL, and LDL/HDL ratio were improved significantly in uremic diabetic patients. These findings illustrate that a 1:1 mixture of EGCG/AE can reduce the risk of complications associated with diabetes and atherosclerosis in uremic diabetic patients. Furthermore, Table 3 and Figure 4 show that changes in an inflammatory marker as well as hepatic and renal functions were statistically insignificant. These results suggest that receiving 1:1 EGCG/AE was safe in the 3-month trial.

This study shows that 1:1 EGCG/AE improves antioxidant defenses, diabetic markers, and atherogenic indices in uremic diabetic patients. In addition, no hepatic or renal toxicity and no inflammatory response were observed during the experimental period. These findings suggest strongly that 1:1 EGCG/AE has potential for use in the treatment of uremic patients with diabetes.

ACKNOWLEDGMENTS

This study is supported in part by the Taiwan Department of Health Clinical Trial and the Research Center of Excellence (grant DOH99-TD-B-111-004).

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

REFERENCES

1. Kormoczi GF, Rosenkranz AR, Zlabinger GJ: Polymorphonuclear granulocyte stimulation by cellulose-based hemodialysis membranes. *Clin Chem Lab Med* 1999;37:351–355.
2. Haag-Weber M, Schollmeyer P, Horl WH: Neutrophil activation during hemodialysis. *Adv Exp Med Biol* 1989;260:29–37.
3. Koken T, Serteser M, Kahraman A, Gokce C: Oxidative stress markers in hepatitis C infected hemodialysis patients. *J Nephrol* 2002;15:302–307.
4. Horl WH: Iron therapy for renal anemia: how much needed, how much harmful? *Pediatr Nephrol* 2007;22:480–489.
5. Sengoelge G, Sunder-Plassmann G, Horl WH: Potential risk for infection and atherosclerosis due to iron therapy. *J Ren Nutr* 2005;15:105–110.
6. Himmelfarb J: Oxidative stress in hemodialysis. *Contrib Nephrol* 2008;161:132–137.
7. Noordzij MJ, Lefrandt JD, Smit AJ: Advanced glycation end products in renal failure: an overview. *J Ren Care* 2008;34:207–212.
8. Meerwaldt R, Zeebregts CT, Navis G, Hillebrands JL, Lefrandt JD, Smit AJ: Accumulation of advanced glycation end products and chronic complication in ESRD treated by dialysis. *Am J Kidney Dis* 2009;53:138–150.
9. Higdon JV, Frei B: Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. *Crit Rev Food Sci Nutr* 2003;43:89–143.
10. Frei B, Higdon JV: Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. *J Nutr* 2003;133:3275S–3284S.
11. Rao TP, Sukaguchi N, Juneja LR, Wada E, Yokozawa T: Amla (*Emblica officinalis* Gaertn) extracts reduce oxidative stress in strepozocin-induced diabetic rats. *J Med Food* 2005;8:362–368.
12. Kusirisin W, Scrichairatanokool S, Lertfrakarnnon P, Lailerd N, Suttajit M, Jaikang C, Chaiyasut C: Antioxidant activity, polyphenolic content and anti-glycation effect of some Thai medicinal plants traditionally used in diabetic patients. *J Med Chem* 2009;5:139–147.
13. Bhattacharya A, Chatterjee A, Ghosal S, Bhattacharya SK: Antioxidant activity of active tannoid principles of *Emblica officinalis* (amla). *Indian J Exp Biol* 1999;37:678–680.
14. Othman A, Ismail A, Ghani NA, Adenan I: Antioxidant capacity and phenolic content of cocoa beans. *Food Chem* 2007;100:1523–1530.
15. Nakagawa T, Yokozawa T, Kim YA, Kang KS: Activity of wenpi-tang, and purified constituents of rhei rhizome and glycyrrizae radix against glucose-mediated protein damage. *Am J Chin Med* 2005;33:817–829.
16. Moshage H, Kok B, Huizenga JR, Jansen PLM: Nitrites and nitrates determination in plasma: a critical evaluation. *Clin Chem* 1995;41:892–896.
17. Ohkawa H, Ohishi N, Yagi K: Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Anal Chem* 1979;95:351–358.
18. Chow HH, Ai Y, Alberts DS, Hakim I, Dorr R, Shahi F, Crowell TA, Yang CS, Hara Y: Phase 1 pharmacokinetic study of tea polyphenol following sing-dose administration of epigallocatechin gallate and polyphenon E. *Cancer Epidemiol Biomarkers Prev* 2001;10:55–58.
19. Chen TS, Liou SY, Chang YL: Supplementation of *Emblica officinalis* (amla) extract reduces oxidative stress in uremic patients. *Am J Chin Med* 2009;37:19–25.
20. Tuzcu M, Sahin N, Karatepe M, Cikim G, Kilinc U, Sahin K: Epigallocatechin-3-gallate supplementation can improve antioxidant status in stressed quail. *Br Poult Sci* 2008;49:643–648.

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AU1: Order of Diabetic and Uremic changed because underlying disease is diabetes, not uremia.
AU2: Why * after last two authors? If needed, define * in footnote.
AU3: No * in table body so statistics footnote deleted.