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Inhibitory effect of some selected nutraceutic herbs on LDL glycation induced by glucose and glyoxal

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Abstract

Anti-LDL glycative agents were investigated using aqueous extracts of *Psidium guajava* L. (PE), *Toona sinensis* Roem. (TE), *Momordica charantia* L. (ME) and *Graptopetalum paragugayene* E. Walther (GE). Concentrations of extracts 0.01–0.625 mg/mL, low density lipoprotein (LDL; 100 μg protein/mL) and inducers glucose (400 mM) and glyoxal (2.5 mM) were incubated at 37 °C. Evaluation parameters involved the thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), relative electrophoretic mobility (REM), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capability and total polyphenolic content. Results for anti-TBARS efficiency (in%) were PE (75.77), TE (75.10), ME (68.81) and GE (19.81) at 0.5 mg/mL, respectively, when induced by glucose; 36.68, 35.60, 32.62 and inactive, respectively, by glyoxal. The lag times for CD formation (in min) were: 289 and 125 by PE and TE, respectively, comparing to the control (45). REM was 1.6 with respect to PE (0.1 mg/mL) compared to the control (4.2). PE at 0.01 mg/mL effectively inhibited with 63.45% efficiency on AGEs induced by glucose. We conclude that PE virtually is a potent antiglycative agent, which can be of great value in the preventive glycation-associated cardiovascular and neurodegenerative diseases.

Keywords: *Psidium guajava* L.; *Toona sinensis* Roem.; *Momordica charantia* L.; *Graptopetalum paragugayene* E. Walther; LDL; Glycation; Glucose; Glyoxal

1. Introduction

Diabetes is an increasing worldwide health problem. It is estimated that the prevalence of diabetes is doubling every 10–15 years and that by 2010 there will be 250 million people expected to be affected with diabetes in the world (Mandrup-Poulsen, 2003). Prolonged exposure to hyperglycemia is now recognized a major factor in the pathogenesis of atherosclerosis in diabetes (Schwartz et al., 1992) and many neurodegenerative diseases (Kikuchi et al., 2003). Vascular dysfunction is among the most common complications associated with diabetes, to which chronic hyperglycemia appears to be an important contributor in this process (Baynes and Thorpe, 1999). The non-enzymatic condensation reaction between the reducing sugars, such as glucose and the amino side chains in proteins (also called Maillard reaction or glycation) has been shown to play an important role in the development of chronic complications of diabetes mellitus

Abbreviations: AGEs, advanced glycation end products; AG, aminoguanidine; BHT, butylated hydroxytoluene; BSA, bovine serum albumin; CD, conjugated diene; DPPH, 1,1-diphenyl-2-picrylhydrazyl; EDTA, ethylenediamine tetraacetic acid; GAE, gallic acid equivalent; GE, aqueous extract from *Graptopetalum paragugayene* E. Walther; LDL, low density lipoprotein; MDA, malondialdehyde; ME, aqueous extract from *Momordica charantia* L.; PBS, phosphate buffered saline; PE, aqueous extract from *Psidium guajava* L.; PPTA, phosphotungstic acid; REM, relative electrophoretic mobility; SDS, sodium dodecyl sulfate; TE, aqueous extract from *Toona sinensis* Roem.; TBA, thiobarbituric acid; TBARS, thiobarbituric acid-reactive substances

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(Aronson and Rayfield, 2002). The appearance of intermediates leading to the formation of Amadori compounds (an aldosylamine; initial reaction of an aldose with an amino group results in the so called Schiff's base, which in turn rearranges slowly to produce 1-amino-1-deoxyketose, an aldosylamine) occurs in the early stage of glycation, while in the late stage, advanced glycation end products (AGEs) are irreversibly formed after a complex cascade of repeated reactions such as dehydration, condensation, fragmentation, oxidation and cyclization (Kikuchi et al., 2003).

Currently, vast evidences have indicated that hyperglycemia has both direct and indirect effects to promote lipoprotein oxidation and, as a consequence, atherosclerosis (Schwartz et al., 1992) and neurodegenerations (Kikuchi et al., 2003). Glucose may combine directly with phospholipids located on the surface of low density lipoprotein (LDL) and apo B lysine groups leading to the formation of AGEs that may further facilitate lipid peroxidation. In addition, the autoxidation of glucose and nonenzymatic glycation of proteins can result in the generation of superoxides, which have been implicated in the mechanism of LDL oxidation in vascular cells (Hiro-Omi et al., 2000). Clinical studies have shown that AGEs-ApoB levels are up to four-fold higher in diabetic patients (Aronson and Rayfield, 2002). Thus, glycated LDL are poorly recognized by the specific LDL receptor, and on the contrary, are preferentially recognized by a nonspecific (scavenger) receptor present on human macrophages. The recognition of glycated LDL by the scavenger receptor pathway is thought to promote intracellular accumulation of cholesteryl esters and hence atherosclerosis (Klein et al., 1995).

Psidium guajava L., commonly known as guava belonging to the family Myrtaceae, is an important tropical fruit in Taiwan. Guava leaves are frequently utilized as a folk medicine, as an astringent hemostatic as well as a folk therapeutic in treatment of diabetes and enteritis (Lee, 1986; Iwu, 1993). *Toona sinensis* Roem. (Meliaceae) is widely cultivated in Taiwan and China. Their leaves and stems have long been used for treatments of enteritis, dysentery and itch in the oriental medicine. The crude extracts of *Toona sinensis* were shown to possess anticancer and hypoglycemic effects (Chang et al., 2002). *Momordica charantia* L. (Cucurbitaceae), commonly known as bitter gourd or karela, is a medicinal plant, popularly used in Asia, India, Africa and South America for treating various diseases, such as diabetes and cancers (Ahmed et al., 2001; Virdi et al., 2003; Rotshteyn and Zito, 2004). In contrast, *Graptopetalum paragugayene* (Crassulaceae) is most widely acclaimed remedy for treatment of diabetes and hypertension in Taiwan. Both hyperglycemia and glycation are related to diabetic (Schwartz et al., 1992) and neurodegenerative complications (Kikuchi et al., 2003). While LDL glycation is thought to play an important role in the pathologenesis of many vascular diseases (Kume et al., 1995). The aqueous extracts of *Psidium guajava* L. (PE), *Toona sinensis* Roem. (TE), *Momordica charantia* L. (ME) and *Graptopetalum paragugayene* E. Walther (GE) were all cited to possess hypoglycemic effect. However, much of their pertinent roles associated with hypoglycemic effects and antiglycative activities on LDL still remain unclear. The present paper explores and compares the inhibitory effects of the above selected nutraceutic herbs on the LDL glycation induced by glucose and glyoxal in order to discover their folkloric therapeutic basis.

2. Materials and methods

2.1. Materials and chemicals

Psidium guajava L., *Toona sinensis* Roem, *Momordica charantia* L. and *Graptopetalum paragugayene* E. Walther were purchased at a local market in Taichung, Taiwan, their origins were identified and proved by the Institute of Medical Herb (Taichung, Taiwan). Glucose, glyoxal, AG, BSA, sodium azide, BHT, PPTA, TBA, SDS, EDTA disodium salt dihydrate and potassium bromide were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Ethanol, *n*-butanol and methanol were obtained from E. Merck (Darmstadt, Germany). All reagents used were of analytical grade. Paragon Lipo gel was obtained from Beckman (CA, USA). Bio-Rad kit was obtained from Bio-Rad (CA, USA).

2.2. Sample preparation

Twenty grams *of Psidium guajava*, *Toona sinensis*, *Momordica charantia* and *Graptopetalum paragugayene* were extracted three times with boiling water, 200 mL for each batch for 30 min, respectively. The combined extracts were filtered through Whatman No. 2 filter paper, the filtrate was lyophilized and pulverized. The extracts yields (in %, w/w, on the starting dry base) from *Psidium guajava*, *Toona sinensis*, *Momordica charantia* and *Graptopetalum paragugayene* were 9.05, 5.25, 7.20 and 1.20, respectively.

2.3. LDL preparation

Plasma for LDL isolation was collected from normal healthy fasting human volunteers in tubes containing in each tube 1 mg/mL of EDTA. LDL ($d = 1.019$–1.063 g/mL) was isolated by sequential ultracentrifugation using a Hitachi Ultracentrifuge (Himac CS 150GXL, Hitachi) following the method previously described by Yamanaka et al. (1997) yet with a slight modification in our laboratory. The LDL solution was flushed with N_2, stored at 4 °C, and used within 1 week after preparation. Protein content was measured using a Bio-Rad kit against a BSA standard. As for glycation and oxidation experiments, each 1 mL of LDL was first dialyzed three times against 1 L (1000-fold volume) of PBS (containing 0.01 M phosphate-buffer and 0.15 M NaCl, pH 7.4) in the dark at 4 °C for 24 h.

2.4. Thiobarbituric acid reactive substances (TBARS)

2.4.1. Reference compound

MDA was used as the reference compound, which on reaction with TBA has a molar extinguishing coefficient at 532 nm ($\varepsilon_{532} = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$) (Kerry and Abbey, 1998).