行政院國家科學委員會專題研究計畫 成果報告

生地黃、葛根、黨蔘、北耆及天花粉熱水提取物在糖尿病合併症保健功效之探勘
研究成果報告(精簡版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 96-2320-B-040-032-
執 行 期 間 ：96 年 08 月 01 日至 97 年 07 月 31 日
執 行 單 位 ：中山醫學大學生物醫學科學學系(所)

計 畫 主 持 人：王祖興

計畫參與人員：碩士班研究生-兼任助理人員：黃苑清
大專生-兼任助理人員：陳韻芳
大專生-兼任助理人員：阮筱彧
大專生-兼任助理人員：王詠茹
大專生-兼任助理人員：張雅筑

處 理 方 式 ：本計畫可公開查詢

中 華 民 國 97 年 10 月 31 日

行政院國家科學委員會補助專題研究計畫 ■ 成 果 報 告
□期中進度報告

生地黃、葛根、黨蔘、北耆及天花粉熱水提取物在糖尿病合併症保健功效之探勘

計畫類別：■ 個別型計畫　　□ 整合型計畫
計畫編號：NSC　96－2320－B－040－032－
執行期間：96 年 08 月 01 日 至 97 年 07 月 31 日

計畫主持人：王祖興
共同主持人：
計畫參與人員：黃苑清、陳韻芳、阮筱彧、王詠茹、張雅筑

成果報告類型(依經費核定清單規定繳交)：■精簡報告　□完整報告

本成果報告包括以下應繳交之附件：
□赴國外出差或研習心得報告一份
□赴大陸地區出差或研習心得報告一份
□出席國際學術會議心得報告及發表之論文各一份
□國際合作研究計畫國外研究報告書一份

處理方式：除產學合作研究計畫、提升產業技術及人才培育研究計畫、列管計畫及下列情形者外，得立即公開查詢
□涉及專利或其他智慧財產權，□一年□二年後可公開查詢

執行單位：中山醫學大學生物醫學系

中　華　民　國　　97 年　　10 月　　30 日

中文摘要

糖尿病(Diabetes mellitus)腎病變是造成末期腎臟病，單一最大的原因，糖尿病為我國每年洗腎新增病患的第一主因。如何預防或延緩糖尿病腎病變造尿毒洗腎，則是國人保健非常重要的研究工作。高血糖糖造成腎病變的分子機制，目前仍不清楚，但蛋白質糖基化作用以及在糖基過程中產生的氧化傷害交互作用，被認為是糖尿病多種合併症重要的原因。因此，同時具有抗蛋白質糖基化活及抗氧化活性的物質，應具有防治糖尿病合併症的潛力。本研究的基本目的即是從安全性高的中藥，利用清除自由基能力及抑制熱誘導葡萄糖造成牛血清白蛋白糖基化的指標，篩選具有抗蛋白質糖基化活性及抗氧化活性之樣品，再進一步了可能的作用機制及有效成分。結果發現在 22 種篩選的中藥，同時具有強力抗蛋白質糖基化及自由基清除活性的有 11 種，包括生地黃、川芎、茵陳蒿、白芷、黃芩、牡丹皮、女貞子、菊花、決明子、佩蘭及甘草；單純抗蛋白質糖基化的有 4 種，包括北耆、黨蔘、澤瀉及茯實；五加皮則是唯一具有高自由基清除活性但不具抗抗蛋白質糖基化的中藥。分析中藥萃取物中總酚(phenolics)含量、總皂素(saponins)含量及總多糖(polysaccharides)含量，發現中藥萃取物之總酚含量與其自由基清除活性相關性較高，而總皂素含量則與抗蛋白質糖化糖活性相關性較高。本研究數據提出中藥抗蛋白質糖基化活性至少有部份是來自其抗氧化活性，而且與所含的總酚含量有關，另外中藥中所含的總皂素組成則更具抗蛋白質糖化糖活性的專一性。因此在對抗糖尿引發的合併症上，選擇同時具有抗氧化及抗蛋白質糖化糖活性的中藥，如生地黃，將優於只具有單純抗蛋白質糖化或抗氧化活性的藥物，如黨蔘及北耆等中藥。

關鍵詞：生地黃；葛根；黨蔘；北耆；花粉；抗氧化；抗蛋白質糖基化；抗氧化

英文摘要

Advanced glycation end products and oxidative stress have been implicated in the pathogenesis of diabetic complications. Both are known to interact with each other. Therefore, exploring natural compounds that possess both antioxidant and antiglycation activities might have great therapeutic potential for treating diabetic complications. The main purpose of this study was to evaluate the antiglycation and antioxidant properties of aqueous extracts from 22 traditional Chinese herbs (TCHs). The polysaccharide, polyphenol, and saponin contents of these TCH extracts were also determined. Our results showed that eleven (50%) TCHs possessed high antioxidant and high antiglycation activity, four TCHs possessed high antiglycation and low antioxidant activity, and only one TCH possessed low antiglycation and high antioxidant activity. There was a close correlation between the antioxidant activity of most extracts and their polyphenol contents. In addition, antiglycation activity showed a weaker correlation with saponin content. These results suggest that the antiglycation activities of most extracts can be explained, at least in part, by their combined antioxidant and antiglycation properties.

Keywords: Traditional Chinese herbs; antioxidant activity; antiglycation activity; polyphenols; polysaccharides; saponins

報告內容

Introduction

Advanced glycation end-products (AGEs) are generated in the diabetic milieu as a result of chronic hyperglycemia and enhanced oxidative stress (Nakamura *et al*., 2007; Gul *et al.*, 2007; Yamagishi *et al*., 2008a). Via direct and receptor-dependent pathways, AGEs promote the development and progression of diabetic complications, including neuropathy, nephropathy, and cardiovascular disease (Sugimoto *et al*., 2008; Fukami *et al*., 2008; Yamagishi *et al*., 2008b). AGEs can accumulate at many sites of the body in diabetes, including the heart and large blood vessels. Because the abundance of AGEs has direct relevance to the pathogenesis of diabetic complications, a clear understanding of the factors contributing to AGE formation may help in ameliorating tissue damage. Recent reports have suggested that metal-catalyzed oxidation reactions play a major role in accelerating the rate of AGE formation. Therefore, agents with antiglycation and antioxidant properties may retard the process of AGE formation by preventing further oxidation of Amadori products and metal-catalyzed glucose oxidation. In fact, inhibitors of AGEs that have antioxidant activity may act as preventive agents against diabetic complications.

China has a long history of treating type 2 diabetes with traditional Chinese herbs (TCHs), and many of these have been proved both safe and effective in clinical practice and in animals. Therefore, to identify potential antidiabetic drugs, it may be useful to directly screen TCHs with antiglycation and antioxidant activities. In the present study, we collected 22 TCHs popularly used in the clinical context and prepared aqueous extracts for screening of antiglycation and antioxidant properties. We also determined the total polyphenol, polysaccharide, and saponin contents of the aqueous extracts.

Material and methods

Preparation of the aqueous extracts

All TCHs were purchased from a Chinese drug store (Nanto, Taichung). Dry herbs were minced with a crushing machine (Yu Chi Machinery, Co., Taiwan). To prepare the aqueous extracts used for the in vitro studies, 0.5 g of minced dry herbs was mixed with 10 mL of ultrapure water and stirred for 30 min at room temperature. The mixtures were filtered through Whatman no. 41 filter paper, and the filtrates were transferred to 15-mL conical tubes and spun at 3500 rpm for 30 min at 4°C. The supernatants were collected, aliquoted, and stored at –20°C until used.

ABTS assay

The ABTS assay (Re *et al*., 1999) was used to measure the antioxidant activity of the aqueous extracts. ABTS was dissolved in deionized water to a concentration of 7 mM, and potassium persulfate was added to a concentration of 2.45 mM. The reaction mixture was allowed to stand at room temperature for 16 h in the dark before use. The resulting intensely colored ABTS•+ radical cation was diluted with 0.01 M PBS (phosphate-buffered saline), pH 7.4, to give an absorbance value of ~0.70 at 734 nm. Two microliters of ABTS•+ cation solution was mixed with 50 μL aqueous extract solution (0.12% final concentration) in a disposable microcuvette with a 1 cm path length. Absorbance was measured at 734 nm after reaction for 6 min. The assay was first carried out on glutathione, which served as a standard. Controls without ABTS•+ were used to allow for any absorbance of the extracts themselves. The ABTS scavenging activity was calculated as inhibition % = [1-(test sample absorbance / blank sample absorbance)] x 100.

Antiglycation assay

The antiglycation assay was performed according to the methods reported by Matsuura and colleagues with slight modifications (Matsuura *et al*., 2002). In all experiments, the final reaction volume was 0.5 mL and the reactions were performed in 1.5-mL microcentrifuge tubes. Albumin (4 mg/mL final concentration) was incubated with glucose (400 mM final concentration) in the presence of traditional herbal extracts (3% final concentration), aminoguanidine (positive control), or PBS as the control buffer at the specified concentration. The reaction was allowed to proceed at 60°C for 48 hours. The reaction mixture was allowed to cool at room temperature, and then 0.2 mL reaction mixture was transferred to a new tube and the reaction was stopped by adding 10 μL of 100% (w/v) trichloroacetic acid (TCA). The TCA-added mixture was kept at 4°C for 10 minutes before centrifugation at 15000 rpm. The precipitate was redissolved with 0.8 mL alkaline PBS (pH 10), and the relative amount

of glycated bovine serum albumin (BSA) was immediately determined on the basis of fluorescence intensity by use of a spectro fluorometer F-4500 (Hitachi, Tokyo, Japan). The excitation and emission wavelengths used were at 370 nm and 440 nm, respectively. Results are expressed as percentage inhibition of formation of the glycated protein.

% inhibition = [1- ($F_{BSA + glucose + TCM extract} - F_{BSA + TCM extract}$) / ($F_{BSA + glucose} - F_{BSA}$)] x 100

where *F* is fluorescence intensity.

Determination of total polyphenols

The total content of polyphenols in the aqueous TCH extracts was quantitated by using the Folin-Ciocalteau colorimetric reaction. Aliquots of 0.5 mL of extract were mixed with 150 mL 20% $NaCO_3$ and 50 μL of Folin-Ciocalteau reagent (Sigma) by vortex. Samples were then incubated in darkness at room temperature for 30 min. Absorbance was read at 730 nm by use of a spectrophotometer. Tannic acid was used as a reference standard, and the total polyphenol content was expressed as tannic acid equivalents (TAE, mg/mg extract).

Determination of total polysaccharides

Total polysaccharide contents in aqueous extracts from TCHs were determined by a phenol-sulfuric acid colorimetric assay (Dubois *et al*., 1951). Two milliliters of solution and 10 mL of ethanol were combined in a plastic tube, the sample was centrifuged at 2500 rpm for 30 min, and the supernatant was removed; the precipitate was dissolved in a final volume of 100 mL water. One milliliter of water, 1 mL of 4% (w/v) phenol, and 1.0 mL of concentrated sulfuric acid were combined in a screw-capped test tube, and the sample was kept in a constant temperature water bath at 40°C for 30 min. The samples were then frozen. The absorbance of thawed samples was determined at 490 nm. The polysaccharide content was estimated by comparison with a standard curve generated from the analysis of glucose.

Determination of total saponins

The total saponin content of each aqueous TCH extract was estimated by using the method described by Hiai *et al*. (1976). The extracts (50 mL) were mixed with vanillin (8% w/v, 0.5 mL) and sulfuric acid (72% w/v, 5 mL). The mixture was incubated at 60°C for 10 min and cooled in an ice bath for 15 min, and absorbance was read at 538 nm. Aescin was used as a reference standard, and the total saponin content was expressed as aescin equivalents (AE, μg/mg extract).

Statistical analysis

Data are expressed as the mean ± S.E. from at least three independent experiments. Statistical analysis was performed with commercially available software (SAS Institute Inc., Cary, NC). Data were analyzed by means of Student's *t*-test. A value of $P < 0.05$ was considered to be significant.

Results

ABTS is a stable radical that is often used to evaluate the antioxidant activity of natural compounds (Re *et al*., 1999). The activities of the extracts in scavenging free radicals are shown in Table 1 and Figure 1. The definitions of the abbreviations used for the extracts are also given in Table 1.Twelve extracts (CX, YCH, SDH, BJR, HC, MDP, NJT, JH, PL, WJP, GT, and JMT) showed significant ABTS scavenging activity (% inhibition > 60%). Most TCH extracts with high ABTS scavenging activity also had high antiglycation activity. WJP was the only extract with high ABTS scavenging activity but low antiglycation activity. The extracts of YR, FL, and CBS had low ABTS radical scavenging activity (% inhibition < 5).

Antiglycation activity was determined by thermal glycation of BSA by D-glucose. These results are shown in Figure and Table 1. Fifteen extracts (CX, BC, TSH, YCH, SDH, BJR, TS, HC, MDP, NJT, JH, PL, CS, GT, and JMT) had strong inhibitory activity of AGE formation (% inhibition > 60%). Among these 15 extracts, the extracts of TSH, TS, and CS had low ABTS radical scavenging activity. On the basis of having inhibition percentages of AGE and ABTS+ radical formation higher than 60%, the 22 extracts were categorized into four groups: (1) high antiglycation and high antioxidant activity, (2) high antiglycation and low antioxidant activity, (3) low antiglycation and high antioxidant activity, and (4) low antiglycation and low antioxidant activity. The percentages of extracts in each of the four groups was 50.0%, 18.2%, 4.5%, and 27.3%, respectively. About 68.2% of the extracts had high antiglycation activity, and 54.5% of the extracts had high antioxidant activity.

Total polyphenols, polysaccharides, and saponins were determined by Folin-Ciocalteau, phenol-sulfuric acid**,** and vanillin-sulfuric acid colorimetry assays, respectively. The data for total contents of polyphenols, polysaccharides, and saponins are shown in Table 1. Total polyphenols ranged from 0.3 to 49.5 mg TAE/g dry weight. The average was 9.2 mg TAE/g dry weight. Of the 22 extracts evaluated, six extracts (MDP, PL, WJP, SDH, HC, and YCH) had higher total polyphenol contents than the average value. Total polysaccharides ranged from 0.5 to 175 mg glucose equivalents (GE)/g dry weight and the average was 19.7 mg GE/g dry weight. Of the 22 extracts evaluated, five extracts (PL, SDH, BJ, JMT, and TSH) had higher total polysaccharides than the average value. Note that the total polysaccharide content of the TSH extract was extremely high compared with the other extracts. Total saponins in the extracts ranged from 0.9 to 121.7 mg AE/g dry weight and the average was 37.9 mg AE/g dry weight. Of the 22 extracts evaluated, nine extracts (BC, NJT, MDP, JH, GT, WJP, SDH, TSH, and YCH) had higher total saponins than the average value. Of the 22 extracts, SDH was the only one with high total polyphenols, polysaccharides, and saponins simultaneously. It also had very good antiglycation and antioxidant

activities compared with the other extracts studied.

Previous studies showed that there is a good linear correlation between total polyphenols and antioxidant activity (Malencić *et al*., 2008; Alali *et al*., 2007; Kiselova *et al*., 2006; Maksimović *et al*., 2005). We used a linear regression analysis to determine whether there was any correlation between antiglycation and antioxidant activity and total polyphenols, polysaccharides, and saponins in extracts. The correlations between antiglycation activity and total polyphenols, polysaccharides, and saponins in extracts are shown in Figure 2 A-C. Although the linear regression analysis indicated a low correlation between antiglycation activity and total polyphenols, polysaccharides, and saponins, the correlation coefficient between total saponins and antiglycation activity was 0.159, which was 1.9-fold and 2.9-fold the correlation coefficient between antiglycation activity and total polyphenols and polysaccharides, respectively. The correlation between antioxidant activity and total polyphenols, polysaccharides, and saponins in extracts is shown in Figure 2 D-F. Although the linear regression analysis indicated a low correlation, the correlation coefficients between antioxidant activity and total polyphenols and total saponins were 0.338 and 0.267, respectively, which were 26-fold and 20.5-fold the correlation coefficient between total polysaccharides and antioxidant activity.

Discussion

In this study, glucose-induced BSA glycation was under a high-temperature condition (60°C). Under such circumstances, the rate of BSA glycation was potently enhanced by glucose. The glycation at physiological 37°C proceeded at a much slower rate than at 60°C; thus, the incubation period was prolonged to at least 14 days (Choi *et al*., 2008; Li *et al*., 2008; Farsi *et al*., 2008; Xi *et al*., 2008). This condition may directly restrict the antiglycation activity of extracts used at physiological 37°C. Although we did not run the same experiment at physiological 37°C, a recent report suggests that 60°C did not affect the antiglycation level of a typical antiglycation agent, aminoguanidine (Jesdsadayammata, 2005). This study also clearly showed that the thermal-catalyzed formation of AGEs is a good rapid method for defining the antiglycation activity of plant extracts *in vitro*.

The plant extracts used for screening samples in this study contained different amounts of natural materials. Intrinsic fluorescence and fluorescence quenching by these natural materials may affect the detection of fluorescent AGE formation, especially when the fluorescent AGE generation is very low (Matsuura *et al*., 2002). In general, the extract samples had significant background fluorescence intensity, and the levels were further increased when they were co-incubated with BSA at 60°C for 48 hours(Figure 3). This increase in fluorescence intensity may have been due to direct binding of the autofluorescent materials to BSA or may have been indirectly induced by the intrinsic sugars in the extracts. Most likely, the components of the extracts were denatured, and excessive amounts of the interfering substances were found under the reaction conditions. Although these interferences can be removed by the TCA precipitation procedure in the assay method of Matsuura that we adopted in this study, the autofluorescent material in the extract samples was not be completely removed. However, the background fluorescence derived from the extract alone or that co-incubated with BSA did not influence the determination of AGE formation by glucose in this study, because background fluorescence from the appropriate control was subtracted. As a result, effective inhibition activity was detected in 71.4% (15/22) of the TCH extracts. This phenomenon indicates that Matsuura's assay is an easy and reliable method for protein glycation inhibitor screening. Even when TCA precipitation to remove the interfering substances was used, the antiglycation inhibition % of several TCH extracts was higher than 100%. Thus, we could not totally discount the false positive that comes from the quenching effect of the TCH extracts on the fluorescence intensity of AGEs-BSA. Further refinement of this assay is needed to eliminate the false positives due to the fluorescence quenching by the TCH extracts.

Previous studies have shown that there is a close correlation between total polyphenols in natural extracts and their antioxidant activity *in vitro* (Lamien-Meda *et*

al., 2008; Kiselova *et al*., 2006) In addition, scattered reports indicate a positive relation between antioxidant activity and total saponins or polysaccharides in the natural extracts (Wang *et al*., 2007; Rodrigues *et al*., 2005). In order to evaluate the possible compositions that are responsible for antiglycation or antioxidant activity in the extracts, we analyzed the total contents of polysaccharides, polyphenols, and saponins by use of standard methods. Although the correlation data were not strong enough to conclude which composition is unequivocally correlated to antiglycation or antioxidant activity, the partial positive correlations between total saponin contents and antiglycation and antioxidant activity were clearly higher than any other comparative groups. Saponins, from a variety of sources, have been shown to have hypoglycemic and antioxidant activity (Francis *et al*., 2002; Rao & Gurfinkel, 2000). Investigators even consider saponins and polyphenols as the key ingredients responsible for most of the observed biological effects of traditional Chinese medicine remedies (Liu & Henkel, 2002). Recent clinical trials also found that combined therapy of total saponins with sulfonylureas as a hypoglycemic drug could lower the blood glucose level and ameliorate clinical symptoms in the treatment of type II diabetic patients whose blood glucose levels were not well controlled by oral hypoglycemic agents; furthermore, the therapy was relatively safe (Lu *et al*., 2008). In addition, a recent report showed that the antioxidant and antiglycation activities of 11 antidiabetic TCHs were positively correlated with the extracted total saponins (Xi *et al*., 2008). By using similar analytical methods, including free radical scavenging and the BSA-glucose assay, Xi and his colleagues also found that *Acanthopanax senticosus* has potent antioxidant and antiglycation activity, similar to our finding in this study. However, it is important to point out that even though the saponin fraction in the antibiabetic TCHs might contain the major active compounds, the purification, chemical structure, and pharmacological activities of these saponins need further investigation. Investigations of the antioxidant and antiglycation activity of these extracts at the mammalian cell level are ongoing in our laboratory.

In this study, 15 extracts had strong antiglycation activity, and 12 extracts also had strong antioxidant activity. The biological functions of these 15 extracts have been widely studied. Most such studies were analyses of antioxidant activity; fewer studies have been conducted on antiglycation/antidiabetes activity. In fact, for these 15 extracts, the overall number of articles collected in the Medline database is 2,064, of which only 3.1% are investigations related to antiglycation/antidiabetes activity (Table 2). Six extracts (BC, GT, JMT, NJT, HC, and SDH) with potent antioxidant or antiglycation activity in the present study were matched with previous other studies; however, the antiglycation or antidiabetes activity of about two-thirds of the extracts in the present study has not been studied. This phenomenon indicates that our findings

are reliable and also have provided some good TCH candidates suitable for antiglycation/antidiabetes investigations.

In conclusion, we evaluated 22 TCHs for their antiglycation and antioxidant activities. The results showed that extracts derived from 15 species showed potent inhibition of glucose-induced glycation of BSA at 60℃ *in vitro*. Among these extracts with high antiglycation activity, 11 also had potent antioxidant activity (Table 1). The extracts with both potential antiglycation and antioxidant activity were derived from CX, YCH, SDH, BJR, HC, MDP, NJT, JH, JMT, PL, and GT. Correlation analysis between the phytochemical composition of these extracts and their antiglycation and antioxidant activity indicated that the total saponin content of the extracts may be responsible for their antiglycation and antioxidant activities. Thus, the total saponins of these 15 TCH species with high antiglycation and high antioxidant activity may be good candidates for further investigation of antidiabetic effects.

References

Abdel-Hassan, I.A., Abdel-Barry, J.A., Tariq Mohammeda, S., 2000. The hypoglycaemic and antihyperglycaemic effect of citrullus colocynthis fruit aqueous extract in normal and alloxan diabetic rabbits. J. Ethnopharmacol. 71, 325-330.

Alali, F.Q., Tawaha, K., El-Elimat, T., Syouf, M., El-Fayad, M., Abulaila, K., Nielsen, S.J., Wheaton, W.D., Falkinham, J.O. 3rd, Oberlies, N.H., 2007. Antioxidant activity and total phenolic content of aqueous and methanolic extracts of Jordanian plants: an ICBG project. Nat. Prod. Res. 21, 1121-1131.

Choi, S.Y., Jung, S.H., Lee, H.S., Park, K.W., Yun, B.S., Lee, K.W., 2008. Glycation inhibitory activity and the identification of an active compound in *Plantago asiatica* extract. Phytother. Res. 22, 323-329.

Dubois, M., Gilles, K., Hamil, J.K., Rebers, P.A., Smith, F., 1951. A colorimetric method for the determination of sugars. Nature 168, 167.

Francis, G., Kerem, Z., Makkar, H.P., Becker, K., 2002. The biological action of saponins in animal systems: a review. Br. J. Nutr. 88, 587-605.

Fukami, K., Yamagishi, S., Ueda, S., Okuda, S., 2008. Role of AGEs in diabetic nephropathy. Curr. Pharm. Des. 14, 946-952.

Gul, A., Rahman, M.A., Salim, A., Simjee, S.U., 2008. Advanced glycation end products in senile diabetic and nondiabetic patients with cataract. J. Diabetes Complications [in press].

Hiai, S., Oura, H., Nakajima, T., 1976. Color reaction of some sapogenins and saponins with vanillin sulfuric acid. Planta Medica 29, 116–122.

Jedsadayanmata, A., 2005. *In Vitro* Antiglycation Activity of Arbutin. Naresuan Univ. J. 13, 35-41 35

Kiselova, Y., Ivanova, D., Chervenkov, T., Gerova, D., Galunska, B., Yankova, T., 2006. Correlation between the in vitro antioxidant activity and polyphenol content of aqueous extracts from Bulgarian herbs. Phytother. Res. 20, 961-965.

Lamien-Meda, A., Lamien, C.E., Compaoré, M.M., Meda, R.N., Kiendrebeogo, M., Zeba, B., Millogo, J.F., Nacoulma, O.G., 2008. Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso. Molecules 13,,581-594.

Li, W., Ota, K., Nakamura, J., Naruse, K., Nakashima, E., Oiso, Y., Hamada, Y., 2008. Antiglycation effect of gliclazide on in vitro AGE formation from glucose and methylglyoxal. Exp. Biol. Med. (Maywood) 233, 176-179.

Liu, J., Henkel, T., 2002. Traditional Chinese medicine (TCM): are polyphenols and saponins the key ingredients triggering biological activities? Curr. Med. Chem. 9, 1483-1485.

Lu, F.R., Shen, L., Qin, Y., Gao, L., Li, H., Dai, Y., 2008. Clinical observation on trigonella foenum-graecum L. total saponins in combination with sulfonylureas in the treatment of type 2 diabetes mellitus. Chin. J. Integr. Med. 14, 56-60.

Maksimović, Z., Malencić, D., Kovacević, N., 2005. Polyphenol contents and antioxidant activity of Maydis stigma extracts. Bioresour. Technol. 96, 873-877.

Malencić, D., Maksimović, Z., Popović, M., Miladinović, J., 2008. Polyphenol contents and antioxidant activity of soybean seed extracts. Bioresour. Technol. 99, 6688-6691.

Matsuura, N., Aradate, T., Sasaki, C., Kojima, H., Ohara, M., Hasegawa, J., 2002. Screening system for the Maillard reaction inhibitor from natural product extracts. J. Health Sci. 48, 520-526.

Nakamura, K., Yamagishi, S.I., Matsui, T., Adachi, H., Takeuchi, M., Imaizumi, T., 2007. Serum levels of soluble form of receptor for advanced glycation end products (sRAGE) are correlated with AGEs in both diabetic and non-diabetic subjects. Clin. Exp. Med. 7, 188-190.

Rao, A.V., Gurfinkel, D.M., 2000. The bioactivity of saponins: triterpenoid and steroidal glycosides. Drug. Metabol. Drug Interact. 17, 211-235.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26, 1231-1237.

Rodrigues, H.G., Diniz, Y.S., Faine, L.A., Galhardi, C.M., Burneiko, R.C., Almeida, J.A., Ribas, B.O., Novelli, E.L., 2005. Antioxidant effect of saponin: potential action of a soybean flavonoid on glucose tolerance and risk factors for atherosclerosis. Int. J. Food Sci. Nutr. 56, 79-85.

Sugimoto, K., Yasujima, M., Yagihashi, S., 2008. Role of advanced glycation end products in diabetic neuropathy. Curr. Pharm. Des. 14, 953-961.

Wang, J., Zhang, Q., Zhang, Z., Li, Z., 2008. Antioxidant activity of sulfated polysaccharide fractions extracted from Laminaria japonica. Int. J. Biol. Macromol. 42, 127-132.

Xi, M., Hai, C., Tang, H., Chen, M., Fang, K., Liang, X., 2008. Antioxidant and antiglycation properties of total saponins extracted from traditional Chinese medicine used to treat diabetes mellitus. Phytother. Res. 22, 228-237.

Yamagishi, S., Nakamura, K., Matsui, T., Ueda, S., Noda, Y., Imaizumi, T., 2008a. Inhibitors of advanced glycation end products (AGEs): potential utility for the treatment of cardiovascular disease. Cardiovasc. Ther. 26, 50-58.

Yamagishi, S., Ueda, S., Matsui, T., Nakamura, K., Okuda, S., 2008b. Role of advanced glycation end products (AGEs) and oxidative stress in diabetic retinopathy. Curr. Pharm. Des. 14, 962-968.

Figure legends

Figure 1. Antiglycation and antioxidant activities of aqueous extracts derived from traditional Chinese herbs. Percent inhibition (%) was determined for antioxidant activity and antiglycation activity at a concentration of 0.12% and 3%, respectively. Experiments were conducted as described under “Materials & Methods”. Values are the mean from at least three independent experiments. The abbreviations used are defined in Table 1.

Figure 2. Antiglycation and antioxidant activities according to the total contents of polyphenols, polysaccharides, and saponins of aqueous extracts derived from 22 different traditional Chinese herbs. Antiglycation activity versus total polyphenols (A), polysaccharides (B), and saponins (C); antioxidant activity versus total polyphenols (D), polysaccharides (E), and saponins (F).

Figure 3. Autofluorescence of extracts from traditional Chinese herbs in the absence or presence of bovine serum albumin incubated at 60°C for 48 hours. After incubation, fluorescence intensity was determined by antiglycation assay as described in “Material & Methods”. Values are the mean from three independent experiments. The abbreviations used are defined in Table 1.

Table 1. Antiglycation and antioxidant activities and composition of aqueous extracts derived from traditional Chinese herbs (TCHs).

TCH	AntiG[d] activity (%)	AntiO[e] activity (%)	TPP[f] (mg TAE[i]/g DW)	TPS[g] (mg GE[j]/g DW)	TS[h] (mg AE[k]/g DW)
ChuanXiong[a]/CX[b] *Ligusticum chuanxiong*[c]	149.2 ± 16.1	100.7 ± 2.3	8.3 ± 0.6	6.1 ± 0.6	33.1 ± 0.7
BeiChi/BC *Astragalus membranaceus*	131.7 ± 20.0	39.1 ± 11.2	4.3 ± 0.3	3.9 ± 0.3	39.7 ± 1.8
TangShen/TS *Codonopsis pilosula*	130.1 ± 6.3	24.7 ± 1.1	1.7 ± 0.1	175.0 ± 4.5	97.4 ± 0.3
YinChenHau/YCH *Artemisia capillaries*	120.5 ± 4.2	101.1 ± 0.1	49.5 ± 1.0	10.1 ± 0.3	121.7 ± 4.0
ShengDiHuang/SDH *Rehmannia glutinosa*	110.9 ± 19.1	100.6 ± 0.5	17.1 ± 0.1	32.1 ± 3.8	90.8 ± 0.5
BaiJr/BJR *Angelicae dahuricae*	104.5 ± 24.9	94.9 ± 1.6	3.8 ± 0.2	6.0 ± 0.4	15.4 ± 0.1
TzeShie/TSH *Alisma orientalis*	100.2 ± 39.6	38.3 ± 1.0	2.1 ± 0.3	3.3 ± 0.2	21.9 ± 0.8
HuangChin/HC *Scutellaria baicalensis*	100.0 ± 10.8	97.3 ± 0.2	40.4 ± 4.0	8.8 ± 0.7	24.6 ± 0.7
MuDanPi/MDP *Paeonia suffruticosa*	99.7 ± 9.4	101.1 ± 1.5	10.2 ± 0.1	2.4 ± 0.6	49.2 ± 3.5
NiuJenTz/NJT *Ligustrum lucidum*	91.5 ± 26.0	99.3 ± 1.7	7.7 ± 0.2	7.0 ± 0.7	41.9 ± 5.0
JiuHua/JH	88.0 ± 6.6	81.2 ± 1.6	8.9 ± 0.3	15.1 ± 2.9	56.6 ± 9.7

Chrysanthemum morifolium					
JiueMingTz/JMT *Cassia obtusifolia*	76.4 ± 1.1	81.2 ± 0.6	3.5 ± 0.7	53.1 ± 0.4	15.5 ± 5.5
PeiLan/PL *Eupatorium fortunei*	69.6 ± 19.1	100.1 ± 0.5	11.1 ± 0.7	29.5 ± 1.7	27.6 ± 7.5
ChianShr/CS *Euryale ferox*	62.1 ± 4.5	16.1 ± 2.2	0.7 ± 0.0	2.2 ± 0.5	1.6 ± 0.1
GanTsau/GT *Glycyrrhiza uralensis*	60.4 ± 0.9	91.3 ± 8.6	7.7 ± 0.0	6.7 ± 0.2	66.5 ± 4.8
YiRen/YR *Coix lacrymajobi*	48.6 ± 5.6	3.4 ± 0.4	0.5 ± 0.0	6.4 ± 1.6	4.4 ± 0.1
ChuanBanShia/CBS *Pinella ternate*	42.0 ± 8.2	0.8 ± 0.6	0.6 ± 0.0	10.1 ± 0.3	1.2 ± 0.0
TianHuaFen/THF *Radix Trichosanthis*	41.4 ± 1.1	32.8 ± 0.5	1.2 ± 0.1	1.6 ± 0.2	3.1 ± 0.2
BaiJu/BJU *Atractylodes macrocephala*	14.0 ± 0.2	29.1 ± 0.6	1.5 ± 0.1	41.3 ± 3.5	28.9 ± 9.9
GeGen/GG *Radix Puerariae*	2.2 ± 25.3	32.7 ± 4.9	4.6 ± 0.2	1.9 ± 0.7	9.3 ± 2.3
WuJiaPi/WJP *Periploca sepium*	-9.5 ± 1.5	97.4 ± 0.9	16.3 ± 0.6	9.6 ± 1.9	81.4 ± 1.4
FuLing/FL *Poria cocoa*	-118.3 ± 52.8	0.7 ± 2.0	0.3 ± 0.0	1.5 ± 0.9	0.9 ± 0.0

a. Chinese name; b: abbreviation; c: scientific name; d: antiglycation; e: antioxidant; f: total polyphenols; g: total

polysaccharides; h: total saponins; i: tannic acid equivalent; j: glucose equivalent; K: aescin equivalent.

Table 2. The status of investigations of the antioxidant, antiglycation, and antidiabetic activity of 15 species of traditional Chinese herbs with high antiglycation activity in the present study.

Chinese name (abbreviation) & Scientific Name	Total Publications (PubMed)	Antiglycation Activity	Antioxidant Activity	Antidiabetic Activity
ChuanXiong (CX) *Ligusticum chuanxiong*	94	0	7	0
BeiChi (BC) *Astragalus membranaceus*	495	3	43	28
TangShen (TSH) *Codonopsis pilosula*	49	0	4	0
YinChenHau (YCH) *Artemisia capillaris* *Artemisia scoparia*	84	0	12	3
ShengDiHuang (SDH) *Rehmannia glutinosa*	157	0	18	11
BaiJr (BJR) *Angelicae dahuricae*	29	0	2	0
TzeShie (TS) *Alisma orientale*	49	0	5	3
HuangChin (HC) *Scutellaria baicalensis*	345	0	110	2
MuDanPi (MDP) *Paeonia suffruticosa*	62	0	13	2
NiuJenTz (NJT) *Ligustrum lucidum*	127	0	22	3

JiuHua (JH) *Chrysanthemum morifolium*	91	0	5	0
JiueMingTz (JMT) *Cassia obtusifolia* *Cassia tora*	80	2	8	1
PeiLan (PL) *Eupatorium fortunei*	4	0	0	0
ChianShr (CS) *Euryale fero*	11	0	3	0
GanTsau (GT) *Glycyrrhiza uralensis* *Glycyrrhiza glabra* *Glycyrrhiza inflata*	387	2	54	6
Total	2064 (100%)	7 (0.3%)	306 (14.8%)	59 (2.8%)

Figure1

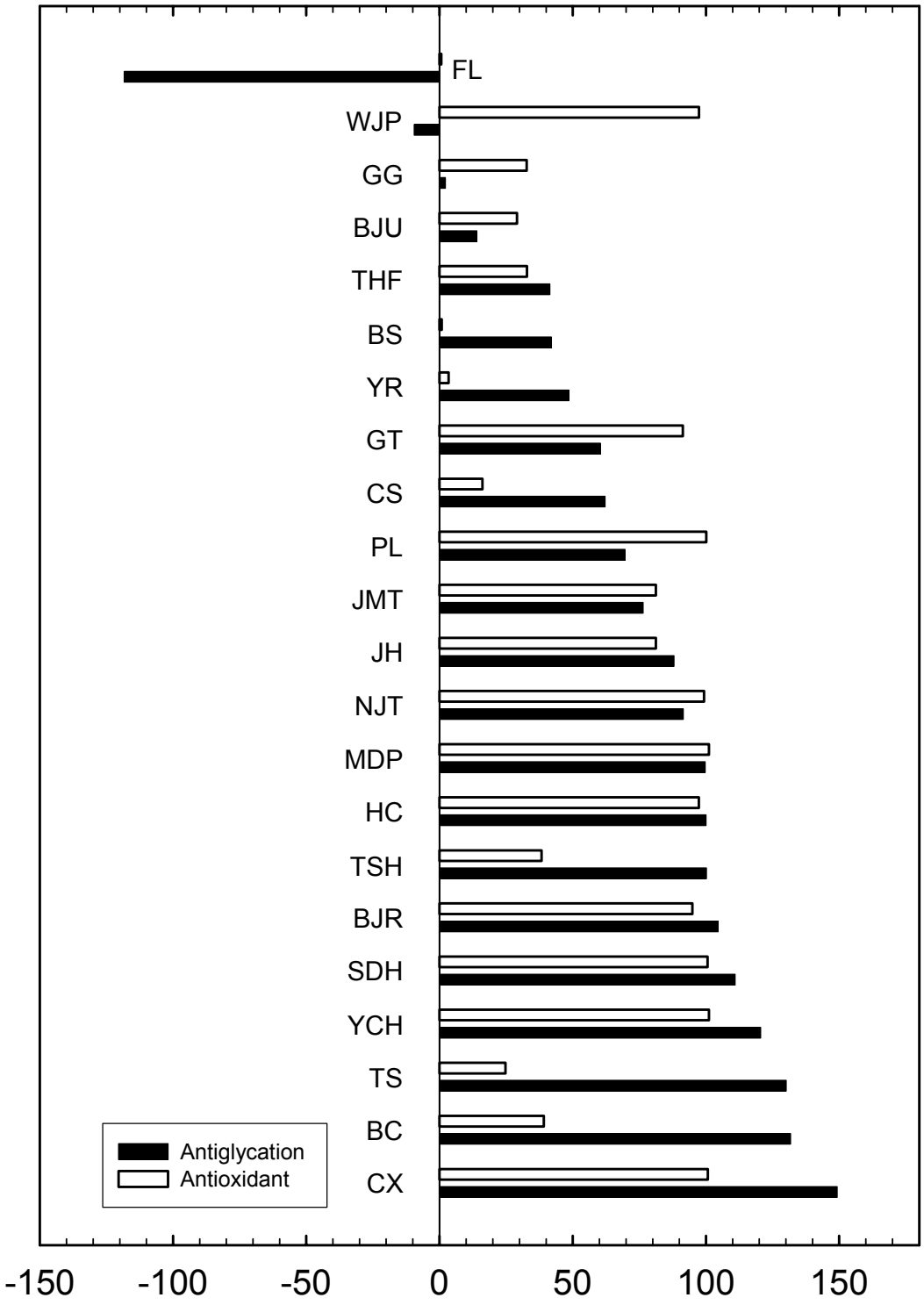
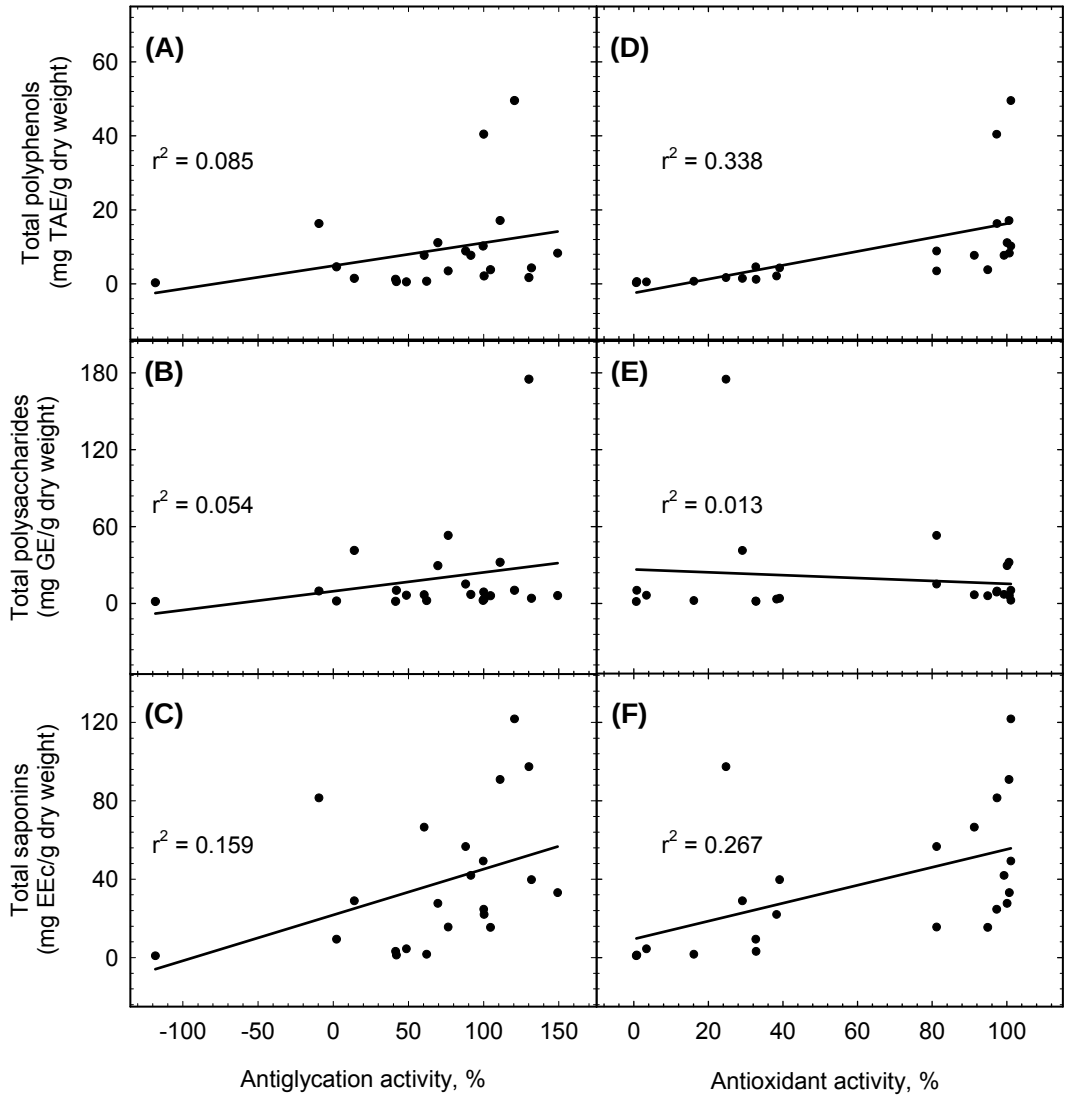
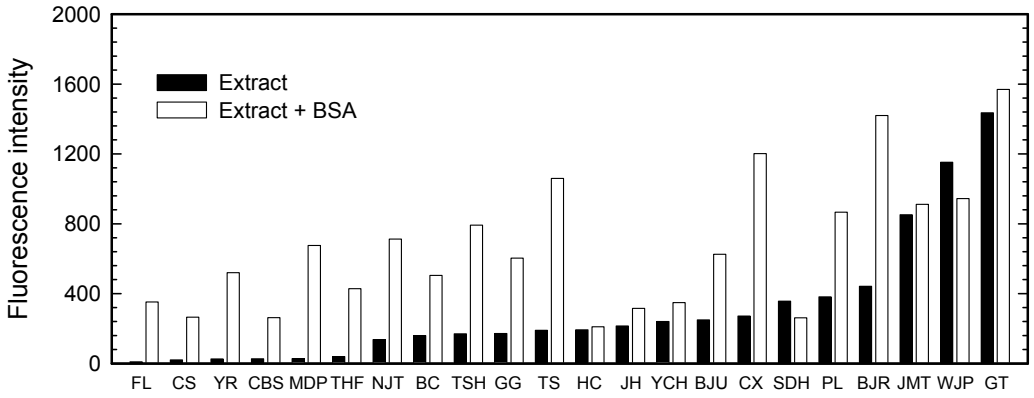

FL
WJP
GG
BJU
THF
BS
YR
GT
CS
PL
JMT
JH
NJT
MDP
HC
TSH
BJR
SDH
YCH
TS
BC
CX
Antiglycation
Antioxidant
-150
-100
-50
0
50
100
150

Figure 2


(A)
(B)
(C)
(D)
(E)
(F)
r2 = 0.085
r2 = 0.054
r2 = 0.159
r2 = 0.338
r2 = 0.013
r2 = 0.267
Total polyphenols (mg TAE/g dry weight)
Total polysaccharides (mg GE/g dry weight)
Total saponins (mg EEc/g dry weight)
Antiglycation activity, %
Antioxidant activity, %


Fluorescence intensity
2000
1600
1200
800
400
0
Extract
Extract + BSA
FL
CS
YR
CBS
MDP
THF
NJT
BC
TSH
GG
TS
HC
JH
YCH
BJU
CX
SDH
PL
BJR
JMT
WJP
GT

成果自評

本研究由原本二年的計畫構想，但僅獲一年的補助，因此在研究內容上也作了一些調整，原則上我們增加了篩選的中藥材數目，並著重這些中藥材組成分與其抗氧化及抗蛋白質糖基化活性間相關性，研究內容與原計畫書大致吻合。研究主要發現(1)中草藥中多酚組成與其抗氧化呈正相關；皂素組成則與其抗蛋白質糖基化活性呈較佳的相關性。(2)中藥對葡萄糖誘導及甲基乙二醛(methylglyoxal)蛋白質糖基化呈現不同程度的差異性，例如黨蔘及北耆只能保護葡萄糖誘導之蛋白質糖基化，對甲基乙二醛誘導之蛋白質糖基化則無保護作用；生地黃卻可同時保護葡萄糖及甲基乙二醛誘導之蛋白質糖基化則無保護作用。(3)大部份中藥都會造成一定程度之蛋白質糖基化背景，在臨床應用上需審慎使用。本研究成果對進一步純化中藥北耆及黨蔘皂素成份，了解在動物模式下的實際效能，可提供重要之參考依據。