Full Length Research Paper

Effect of guava (Psidium guajava Linn.) fruit water extract on lipid peroxidation and serum lipid profiles of streptozotocin-nicotinamide induced diabetic rats

Hsueh-Chin Chao1, Po-Hua Wu2, Diana Lo1, Wan-Ting Wu1 and Ming-Chang Wu1*

1Department of Food Science, National Pingtung University of Science and Technology, No.1 Hsueh Fu Road, Nei-Pu Hsiang, Pingtung 91201, Taiwan.
2Department of Microbiology, Immunology, and Biopharmaceuticals, National Chiayi University, No.300 Syuefu Road, Chiayi City 60004, Taiwan.

Accepted 27 August, 2013

The prevalence of diabetes is rapidly increasing and it can cause a wide range of health complications affecting almost every part of the body. The aim of this study was to examine guava fruit ability in reducing blood glucose level and preventing diabetic complication of streptozotocin-nicotinamide induced diabetes rat. Guava fruit performance was measured by in vitro lipid peroxidation inhibition, in vivo blood glucose level, in vivo serum lipid profile, and in vivo lipid peroxidation inhibition. This study confirmed that guava fruit extracts can significantly reduce blood sugar in type 2 diabetic rats, plasma triglycerides and low density lipoprotein (LDL) concentrations. Most importantly, guava fruit water extract showed impressive efficacy against in vitro and in vivo lipid peroxidation examinations. These activities may be due to the antioxidants properties offered from phenolic and flavonoid compounds in guava fruit water extract. Guava fruit water extract was found to consist 40.13 mg GAE/g of phenolic content and 18.43 ± 1.22 mg quercetin equivalent/g of flavonoid content.

Key words: Guava fruit, lipid peroxidation, malonaldehyde, serum lipid profile, diabetes mellitus.

INTRODUCTION

Due to the economic conditions improvement, various types of health-oriented food products have gradually been paying attention and diabetic health food is not an exception. The prevalence of diabetes is rapidly increasing in industrialized countries, and type 2 diabetes accounts for 90% of the disease. Diabetes can cause a wide range of health complications, affecting almost every part of the body. However, many of these complications can develop and become quite severe before the patient even realizes there is a problem brewing; hence the reason diabetes can be called a silent killer among diseases.

Most of western medicines, which are often made of a single chemical compound, are very effective for direct relief of symptoms, such as lowering blood sugar. Chinese medicines are effective not only to treat and prevent diabetic complications but also at the meantime to lower blood glucose level (Li et al., 2004). Therefore,
Chinese doctors often make a combination of traditional medicine with Western medicine, Western medicine for reducing blood sugar, traditional medicine for integrated care of body (Cheng et al., 1998).

Previous studies have found that free radical activity, lipid peroxidation products accumulation and also oxidative stress caused by unbalance antioxidant defense system seems to have important role in diabetes and its complications (Oberly, 1988; Baynes, 1991). Lipid accumulation in the liver has been shown to play a key role in insulin resistance, and alleviation of this condition was suggested to be one mechanism to improve insulin sensitivity (Kim et al., 2001). On the other hand, dyslipidemia (abnormal amounts of serum lipids), associated with insulin resistance can increase the risk for cardiovascular disease (CVD) of diabetic complication (Goldberg et al., 2005).

White guava (Psidium guajava L.), as one of traditional Chinese medicines, it is widely cultivated and mostly consumed fresh. Guava leaves, fruit and stem bark were also used as a hypoglycemic agent in folk medicine. Hypoglycemic activity of guava leaves has been well-documented (Maruyama et al., 1985; Shen et al., 2008; Cheng et al., 2009), but not for guava fruit. Cheng and Yang (1983) has proved that guava juice exhibited hypoglycemic effects in mice by examining blood glucose level. As diabetic complication also contribute to ill health, disability, poor quality of life and premature death, observing the potential of natural product on diabetic complication become important. Therefore, the aim of this study was not only to examine hypoglycemic effect of guava fruit extract but also its ability in preventing diabetic complication by examining in vitro and in vivo lipid peroxidation inhibition and blood lipid profile of streptozotocin-nicotinamide induced diabetes rat.

MATERIALS AND METHODS

Plant and chemicals

Half ripen white guava (P. guajava Linn.) fruits were collected from YanChao Township in Kaoshiung County. Streptozotocin, heparin, bovine serum albumin (BSA), Folin-Ciocalteu’s phenol reagent, and thiobarbituric acid (TBA) were purchased from Sigma (MO, USA). Glucose enzymatic kits test were purchased from Audit Diagnostics, Cork, Ireland. All other reagents were of analytical grade. The instruments used were spectrophotometer (HITACHI U-1100, Tokyo, Japan) and freeze dryer (Panchum, FD-5030, Taiwan).

Sample preparation

Guava fruit were washed with clean water, drained, weighed and cut into slices. Slices were kept inside 80°C oven for dehydration. After drying process, the sample was ground into powder to increase contact surface area during extraction. For extraction procedure, 10 ml distilled water to 1 g dried sample ratio were heated inside 85°C incubator for 30 min. The extract was then cooled to room temperature, filtered and centrifuged at 4°C, 9,000 × g for 30 min in order to separate extracted with non-extracted compound. Afterwards, supernatant were collected and freeze-dried into powder. The powders were kept in -20°C freezer.

Proximate chemical composition analysis

General composition analysis was done to measure freeze dried guava fruit water extract composition. It was divided into six parts; moisture test, water activity, ash content, crude fat, crude protein, and total carbohydrate content based on Association of Analytical Communities (AOAC) (1997).

Phenolic and flavonoid contents determination

Total phenolic content was measured according to the protocol by Kujala et al. (2000) by using 0, 50, 100, 150, 200, 250 µg/ml gallic acid as standard. Sample or standard (0.2 ml) were diluted into 2 ml, and 0.1 ml Folin-Ciocalteu’s phenol reagent were added into sample. Then, 2.5 ml of 20% (w/v) sodium carbonate solution was added and mixture was allowed to react for 20 min. The absorbance was determined at 765 nm by spectrophotometer. Total phenolic contents were represented as mg gallic acid equivalent per gram dry weight sample (GAE). Flavonoid content was measured by colorimetric reaction as described previously by Christel et al. (2000) by using 0 to 100 µg/ml of quercetin as standard. 95% alcohol (1.5 ml) was added into 0.5 ml standard or sample, and several series of regents were added: 0.5 ml of 1 M aluminum nitrate, 0.5 ml of 1 M potassium acetate and 2.8 ml distilled water. After stirring and incubating for 30 min, the absorbance values were measured at 415 nm. Flavonoid contents were shown as mg quercertin equivalent per gram dry weight sample.

In vitro Fe²⁺-ascorbic acid induced lipid peroxidation assay

Five week old male normal Wistar rats were sacrificed and 1 g of liver cell was taken and disrupted with 10 ml of 150 mM Tris-HCl (pH 7.2) buffer using homogenizer, followed by centrifugation at 500 × g for 10 min. Supernatant was harvested and diluted with water to make 10% (w/v) liver cell for lipid peroxidation analysis. Lipid peroxidation analysis was done according to Yoden et al. (1980) and Kimura et al. (1981) method. Obtained liver cell (0.25 ml) was added into test tube and following reagents were added: 0.05 ml Tris-HCl buffer (pH 7.2), 0.05 ml of ascorbic acid (0.1 mM), 0.05 ml of FeCl₂ (4 mM) and 0.05 ml sample (25, 50, 100, 200, 400, 800 µg/ml). Freeze dried water extract of guava (80 mg) was diluted with 100 ml of water to obtain sample with the concentration of 800 µg/ml and two times dilution was conducted to obtain the others concentrations. Solution mixture was incubated at 37°C for 1 h after mixing. After incubation, the mixture were added with 0.5 ml HCl (0.1 N), 0.2 ml sodium dodecyle sulphate (SDS) (9.8%), 0.9 ml distilled water and 2 ml (0.6% thiobarbituric acid; TBA). The mixture was then incubated at 95°C for 30 min. After cooling at room temperature, 5 ml n-BuOH was added with intense oscillation and centrifuged again with 1,000 × g for 25 min. Supernatant was taken and observed using florescence photometer at 515 nm excitation and 553 nm emissions to measured malondialdehyde (MDA) concentration. Negative control was examined using liver solution that was not treated with FeCl₂ and ascorbic acid. Positive control was examined using liver solution that was treated with FeCl₂ and
Table 1. Guava fruit’s proximate chemical composition and phenolic content.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water content</td>
<td>11.77±1.85</td>
</tr>
<tr>
<td>Water activity</td>
<td>0.44±0.02</td>
</tr>
<tr>
<td>ash</td>
<td>17.85±0.16</td>
</tr>
<tr>
<td>fat</td>
<td>0.83±0.18</td>
</tr>
<tr>
<td>Crude protein</td>
<td>16.48±1.32</td>
</tr>
<tr>
<td>Total carbohydrate</td>
<td>35.76±1.74</td>
</tr>
<tr>
<td>Phenolic compound</td>
<td>Content (mg/g)</td>
</tr>
<tr>
<td>Total phenolic compounds</td>
<td>40.13±2.12</td>
</tr>
<tr>
<td>Flavonoids compounds</td>
<td>18.426±1.22</td>
</tr>
</tbody>
</table>

Each value represents mean ± standard deviation (n = 3).

**Lipid peroxidation assay in vivo**

Thiobarbituric acid reactive substances (TBARs) are low molecular weight compounds formed by decomposition of certain primary and secondary lipid peroxidation products that at low pH and high temperature participate in nucleophilic addition reaction with thiobarbituric acid generating red fluorescent complex (Janero, 1990). Determination was done according to Uchiyama and Mihara (1978) method by adding 9 fold (w/v) of cold 1.15% KCl solution into 0.5 g liver and homogenized in ice bath. After homogenization, 3 ml H₂PO₄ (1%) and 1 ml 0.67% thiobarbituric acid (TBA) were added into 0.5 ml of the homogenized liver as sample or 0.9% saline as blank or 1.1.3.3-tetraethoxy (0 to 10 μg/ml) as standard. After incubation in water bath at 95°C for 45 min, 4.0 ml n-butanol were added and centrifuged at 3,000 rpm (1,570 × g) for 10 min. The supernatant were measured at 520 and 535 nm by florescence photometer. Finally, TBARS values were converted from standard curve.

**Statistical methods**

Experiments were done in three replications for in vitro analysis and twelve replications for in vivo analysis. One way analysis of variance followed by post hoc testing (Duncan’s test) was used for statistical analysis where appropriate, at P < 0.05 by using statistical package for social sciences (SPSS).

**RESULTS**

**Proximate chemical general composition and antioxidant content analysis**

The amount of total phenolic content of guava water extracts was 40.13 ± 2.12 mg/g of dry weight sample and the flavonoid content was 18.426 ± 1.22 mg/g of dry weight sample. Proximate chemical general composition of guava leaf water extract is shown in Table 1.

**In vitro anti-peroxidation evaluation**

In this experiment, lipid peroxidation assayed as malondialdehyde (MDA) production, was catalyzed by ascorbic acid and Fe²⁺ and after reacting with TBA, MDA will form a visible red color product. MDA is one of lipid oxidized derivatives and can be used as a biomarker of oxidative stress. As a result, 25 to 800 ppm guava fruit extracts offer dose-dependent protection from lipid peroxidation in vitro. Each concentration was able to effectively reduce MDA formation, and highest inhibition rate was 57.27% by 800 ppm guava fruit extracts (Table.2).

**In vivo evaluation**

Experiments in 0-, 4- and 6-week after guava fruit extract administration were observed and the changes in blood ascorbic acid only (without extract).

**In vivo assay**

**Experimental animals**

The five-week Wistar strain male rats were purchased from the Bio Lasco, Taiwan and the rat chows were purchased from Yong Li Company (Taipei, Taiwan) with compositions: 23% crude protein, 4.5% crude fat, 6.0% crude fiber, 8.0% ash, 2.5% minerals, 56% carbohydrate. The Wistar strain male rats were fed ad libitum. Body records were recorded weekly, and after body weight reached about 200 to 230 g, the rats were ready for experiment. Induction of streptozotocin plus nicotinamide to generate diabetic rat was done by following Masiello et al. (1998) induction methods by intraperitoneal injection using nicotinamide (180 mg/kg BW) and streptozotocin (STZ) (50 mg/kg BW) with 15 min time interval. Second injections were carried out a week later by the same procedure. Two weeks after second injections, oral glucose tolerance tests were conducted and blood glucose was measured at 0, 30, 60, 120 min. After 120 min of sugar feeding (1.5 g/kg BW), rats with blood glucose level > 200 mg/dl were considered to have severe diabetes type 2. After several weeks of sample or deionized water treatments, the rats were sacrificed after 12 h fasting. Blood and liver of rats were taken for analysis. For animal grouping, rats were randomly divided into four groups (n = 12). Normal group (NC): non-diabetic rats with deionized water feeding, control group (DC): diabetic rats with deionized water feeding, experimental group 2 (DF2): diabetic rat with 200 mg/kg BW sample feeding, experimental group 4 (DF4): diabetic rat with 400 mg/kg BW sample feeding. Guava feeding was carried out daily for six weeks.

**Determination of plasma glucose concentration and serum lipid profile**

Blood plasma was taken and 10 μl of plasma was mixed with 1 ml of glucose enzymatic kits test and incubated at 37°C for 5 min. The absorbances were measured by spectrophotometer at 505 nm wavelength and glucose content was converted from glucose standard curve. Blood cholesterol, triglycerides, high density lipoprotein (HDL) and low density lipoprotein (LDL) concentrations were detected using commercial kit.
Table 2. Effect of guava fruit water extract on Fe^{2+}-ascorbic acid induced lipid peroxidation as expressed by MDA amount.

<table>
<thead>
<tr>
<th>Treatment concentrations</th>
<th>MDA (nmol mg^{-1} protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (normal liver)</td>
<td>15.74±0.86^a</td>
</tr>
<tr>
<td>Positive control (untreated with extract)</td>
<td>117.24±2.29^e</td>
</tr>
<tr>
<td>25 µg/ml of guava fruit extract</td>
<td>95.06±10.34^d</td>
</tr>
<tr>
<td>50 µg/ml of guava fruit extract</td>
<td>85.00±4.73^c</td>
</tr>
<tr>
<td>100 µg/ml of guava fruit extract</td>
<td>67.73±3.50^b</td>
</tr>
<tr>
<td>200 µg/ml of guava fruit extract</td>
<td>59.92±10.69^b</td>
</tr>
<tr>
<td>400 µg/ml of guava fruit extract</td>
<td>59.22±6.98^b</td>
</tr>
<tr>
<td>800 µg/ml of guava fruit extract</td>
<td>57.27±2.10^b</td>
</tr>
</tbody>
</table>

Each value represents mean ± standard deviation (n = 3). ^a-d Data with different superscripts are significantly different at P < 0.05 (n = 3) analyzed by ANOVA with Duncan’s post hoc using SPSS.

Figure 1 Effect of guava water extract admission on plasma glucose concentration for 4 and 6 weeks treatment. Each value represents mean ± standard deviation (n = 12). NC, normal control; DC, diabetic control; DF2, diabetic rats fed with 200mg guava water extract per kg body weight; DF4, diabetic rats fed with 400 mg guava water extract per kg body weight. ^a-c Data with different superscripts are significantly different at P<0.05 (n=3) analyzed by ANOVA with Duncan’s post hoc using SPSS.

glucose were recorded (Figure 1). At 0 week, significant damage was evident to DC, DF2 and DF4 rat group which was indicated by higher blood glucose level after streptozotocin injection. The rats in the group which received water extract of guava showed rectification of increased blood glucose level. After 4 weeks of guava extract administration, blood glucose level was found to be decreased significantly, thus indicating that guava extract can reduce plasma glucose concentrations of diabetic rats. After six weeks, blood glucose levels of the DF2 and DF4 groups were found to be lower than 200 mg/dl, and almost ameliorated the damage caused by streptozotocin to normalcy. Changes of serum lipid profile are shown in Table 3. Plasma triglyceride on DF2 group was reduced and significant reduction was expressed by DF4 group. DF2 and DF4 groups showed decline in
Table 3. Effect of guava fruit water extract admission for two weeks on lipid profile.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lipid profile (mg dL⁻¹)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Triglyceride</td>
<td>Cholesterol</td>
<td>HDL</td>
<td>LDL</td>
</tr>
<tr>
<td>NC</td>
<td>53.00 ±6.26ᵃᵇ</td>
<td>67.80±4.46ᵇ</td>
<td>17.80±3.56ᵃ</td>
<td>11.40±3.58ᵃ</td>
</tr>
<tr>
<td>DC</td>
<td>65.00±4.08ᵇ</td>
<td>61.00±1.89ᵃᵇ</td>
<td>24.00±9.63ᵃ</td>
<td>38.50±4.36ᵇ</td>
</tr>
<tr>
<td>DF2</td>
<td>56.20±6.96ᵃᵇ</td>
<td>61.40±1.14ᵃᵇ</td>
<td>22.40±1.67ᵃ</td>
<td>24.20±2.77ᵇ</td>
</tr>
<tr>
<td>DF4</td>
<td>49.25±6.78ᵃ</td>
<td>59.00±6.16ᵃ</td>
<td>23.00±2.45ᵃ</td>
<td>18.00±6.06ᵃᵇ</td>
</tr>
</tbody>
</table>

Each value represents mean ± standard deviation (n = 12). NC, normal control; DC, diabetic control; DF2, diabetic rats fed with 200 mg guava water extract per kg body weight; DF4, diabetic rats fed with 400 mg guava water extract per kg body weight.

ᵃ⁻ᵇ Data with different superscripts are significantly different at P<0.05 (n = 3) analyzed by ANOVA with Duncan’s post hoc using SPSS performed separately for every analysis.

Figure 2. Effect of guava fruit water extract admission on rat liver as expressed by TBARS content. Each value represents mean ± standard deviation (n = 12). NC, normal control; DC, diabetic control; DF2, diabetic rats fed with 200 mg guava water extract per kg body weight; DF4, diabetic rats fed with 400 mg guava water extract per kg body weight.

ᵃ⁻ᵇ Data with different superscripts are significantly different at P<0.05 (n = 3) analyzed by ANOVA with Duncan’s post hoc using SPSS.

Cheng and Yang (1983) have proved that guava juice could reduce blood glucose level in mice and human, and this present study also revealed the same blood glucose lowering ability in rat. Anti-hyperglycemic activity of unripe guava peel also showed similar result (Rai et al., 2009). Its anti-hyperglycemic activity may due to its ability to inhibit α-glucosidase enzyme in intestine which in turn reduce glucose formation. In vitro α-glucosidase inhibition performed by 0.05 mg/ml guava fruit extract was about 70% (Wongsa and Zamaluddien, 2011).

Lipid peroxidation is the process in which free radical steal electron from double-layer polyunsaturated fatty acids on cell membrane, resulting in toxic aldehyde type塞尔 LDL level, especially in DF4 group. Other lipid profiles (cholesterol and HDL) did not show significant difference compared to diabetic control (DC) group. TBARS in the liver are an indicator of damage caused by lipid peroxidation. Figure 2 shows that guava fruit water extract administration on rat can significantly reduce TBARS content that was generated by lipid peroxidation, either in DF2 or DF4 groups compared to DC group.

DISCUSSION

Cheng and Yang (1983) has proved that guava juice could reduce blood glucose level in mice and human, and this present study also revealed the same blood glucose lowering ability in rat. Anti-hyperglycemic activity of unripe guava peel also showed similar result (Rai et al., 2009). Its anti-hyperglycemic activity may due to its ability to inhibit α-glucosidase enzyme in intestine which in turn reduce glucose formation. In vitro α-glucosidase inhibition performed by 0.05 mg/ml guava fruit extract was about 70% (Wongsa and Zamaluddien, 2011).

Lipid peroxidation is the process in which free radical steal electron from double-layer polyunsaturated fatty acids on cell membrane, resulting in toxic aldehyde type...
of lipid peroxides, such as malondialdehyde (MDA). In diabetes, MDA can cause protein glycation that leads to further complications occurrence (Slatter et al., 2000; Pariand Latha, 2005). This study found that a higher dose of guava extract has a better inhibition of liver lipid peroxidation effect in vitro. In vitro evidence of lipid peroxidation inhibition is also supported by its in vivo result. This inhibition may occur due to guava’s antioxidant activity from ascorbic acid and phenolic compounds. According to Soares et al. (2007), mature white guava fruits contained high amount of ascorbic acid, which was about 168.36 mg/100 g samples and it was mostly found in peel part. Phenolic compounds was a group of compounds well-known for its antioxidant properties. Sa’nez-Moreno et al. (1999) indicated that the inhibition of lipid oxidation of the phenolic compounds and antioxidant standards followed the order: rutin, ferulic acid > tannic acid, gallic acid, resveratrol > BHA, quercitin > tocopherol > caffeic acid, in a linoleic acid system. These flavonoids act by scavenging superoxide anions, singlet oxygens and lipid peroxy radicals, and through sequestering metal ions that promote oxyradical formation (Rao, 2003). Since guava fruit water extract consisted of 40.13 mg GAE/g phenolic content and 18.43 ± 1.22 mg quercetin equivalent/g flavonoid content, we suspected that phenolic and flavonoid contents in guava fruit extract may have important role in inhibiting lipid peroxidation. This study found that high doses of guava fruit water extract could also reduce low density lipoprotein (LDL) and triglycerides content in the diabetic rat significantly. Similar research on hypertension patient by Singh et al. (1992) showed that guava fruit intake also caused serum cholesterol (9.9%) and triglyceride (7.7%) reduction and an increase in high-density lipoprotein cholesterol (8.0%). Anti-LDL glycatcative agents investigated using aqueous decoctions of P. guajava ripe fruit revealed that guava fruits exhibit excellent antiglycation effect, being a rather powerful and effective inhibitor of LDL glycation in both glucose and glyoxal induced models (Hsieh et al., 2005). The antiglycation activities of guava fruit were relevantly and directly related to its polyphenolic content. Total grain, whole-grain, total dietary fiber, cereal fiber, and dietary magnesium intakes showed strong inverse associations with incidence of diabetes (Meyer et al., 2000). Pulp and peel of guava fruit showed high content of dietary fiber of 48.55 to 49.42% (Jiménez-Escrig et al., 2001). Dietary fiber was expected to offer some improvement in carbohydrate metabolism, lower total cholesterol and LDL cholesterol, and have other beneficial effects in patients with non-insulin dependent diabetes mellitus (Vinik and Jenkins, 1998).

Beside its dietary fiber content, guava fruits are also rich in magnesium and potassium. From previous research, Singh et al. (1991) stated that it is possible that dietary magnesium may have contributed to the reduction of total serum cholesterol, LDL cholesterol, and triglyceride, and the marginal rise in HDL cholesterol.

Conclusion

This study confirmed that guava fruit extracts can reduce blood sugar in type 2 diabetic rat, plasma triglycerides and LDL concentrations. Integrated role from several compounds contained in guava fruit extract, especially phenolic compounds, was expected to give contribution on these activities. From this research, guava fruit water extract was able to show impressive efficacy against in vitro and in vivo lipid peroxidation, plasma triglycerides and LDL concentration, but also expected not only able in blood glucose reduction but also diabetic complication preventer.

REFERENCES


