Consumption of purple sweet potato leaves decreases lipid peroxidation and DNA damage in humans

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Consumption of polyphenols is associated with reduced risk of chronic diseases, possibly via a variety of bio-mechanisms, including antioxidation and anti-inflammation. Purple sweet potato leaves (PSPL) commonly consumed in Asia possess polyphenols. In this study, we aim to investigate antioxidant effect of 200 g/d PSPL containing 902 mg polyphenols in a clinical trial. This randomized, crossover clinical study included 16 healthy adults (7 M, 9 F; aged 20-22 y). After a 1-wk run period, subjects were assigned randomly to receive either PSPL or low polyphenol diet (LPD) for 2 wks, followed by a 2-wk washout period before crossing over to the alternate diet. Fasting blood and 24-h urine samples were collected from each subject at day 0, 7 and 14 of each phase. Our data showed PSPL consumption enhanced urinary total phenol excretion by 24.5% at day 14 as compared to day 0, while the LPD decreased total phenol content in plasma and urine by 3.3 and 16.3%, respectively (p ≤ 0.05). Low-density lipoprotein lag time and glutathione concentration in erythrocytes at day 14 was significantly enhanced by 15.0 and 33.3% by PSPL as compared to day 0, respectively, while their values were not altered by the LPD. Urinary 8-hydroxy-deoxyguanosine (8-OHdG) excretion decreased significantly by PSPL consumption by 36.7% at day 7 as compared to day 0, yet unchanged by the LPD (p ≤ 0.05). In conclusion, our results suggest that polyphenols in 200 g PSPL were bio-available and could enhance antioxidant defense and decrease oxidative stress in young healthy people.

Key Words: purple sweet potato leaves, polyphenols, lipid peroxidation, 8-hydroxydeoxyguanosine, DNA damage

INTRODUCTION
Evidence from epidemiological studies suggested a strong, inverse association between incidence of chronic diseases and intake of plant foods, possibly due to their high nutrient density and low fat contents.1-5 Thereby, consumption of plant foods has been strongly promoted and promulgated in the dietary guidelines by the public health authorities and regulatory agencies.6 Nevertheless, contribution of nonessential phytonutrients ubiquitous in plant foods to reduced the risk of health problems via an array of putative mechanism of bioactions, including anti-inflammation, antioxidation, anti-proliferation, and induction of phase II enzymes has been gradually recognized.7-9 In particular, there is growing interest in polyphenolic compounds because of their prevalence in plants, as well as potent antioxidant activity.10 Leaves of sweet potato (Ipomoea batatas) have been consumed commonly in Asian countries and are rich in micronutrients.11 Because this plant tolerates well against diseases, pest infestation, and flooding,12 leaves of sweet potato can provide health benefits to people residing in resource poor areas. Like other plant foods, grapes, green tea, onions, these leaves contain polyphenols ranging from 2-14 g/100g dry weight and exhibit antioxidant13,14 and anti-mutagenic activity.15 Recently, we observed in a clinical trial that a 2-wk supplementation of 200 g/d cooked purple sweet potato leaves (PSPL) increased Con A-activated proliferation and IL-2 and -4 secretions in peripheral blood mononuclear cells and elevated lytic activity of NK cells.16 In other human trial, we also found that 200 g/d PSPL for 2 wks enhanced total phenol content in plasma and LDL resistance against oxidation and decreased urinary 8-hydroxydeguanosine (8-OHdG) in elite basketball players.17

While health benefits of polyphenolic compounds could be mediated via a wide spectrum of bioactions, the effect of PSPL incorporated into daily diets on antioxidant defenses and biomarkers of oxidative stress in health individuals remains to be examined. Thus, in this study,
we aim to investigate whether addition of 200 g/d PSPL to a low polyphenol diet (LPD) for 2 wk can enhance antioxidant defenses and thereby decrease oxidative stress in a cross-over clinical trial. The information gathered from this study is useful for promoting inclusion of sweet potato leaves for health promotion and prevention in resource poor areas.

MATERIALS AND METHODS

Preparation of purple sweet potato leaves

Purple sweet potatoes were planted at the Taoyuan District Agriculture Improvement Station, Taipei Branch, Taiwan, which is 1 hour away from the Taipei Medical University. Fresh PSPL were shipped daily to our metabolic research unit, weighted, washed, stir fried in soybean oil, and then provided to subjects.

Subjects

Sixteen non-smokers (7 M, 9 F, age: 20-22 yrs, BMI: 20.6-21.4 kg/m²) in good health condition, based on results from a medical history questionnaire, physical examination, electrocardiogram test, and standard clinical biochemistries. Exclusion criteria included: 1) history of cardiovascular, hepatic, gastrointestinal, and renal disease; 2) alcoholism; 3) use of antibiotics or multi-vitamin and mineral for ≥4 wk prior to the study. Volunteers were asked not to take any vitamin supplement or medication during the whole study period. The study was approved by the Medical Ethical Committee of the Institutional Review Board from Taipei Medical University, and written consent was obtained from each participant.

Study design

A randomized, crossover design was employed in this study. The duration of the whole study was 7 wk, including 1-wk run-in and 2 phases of 2-wk dietary treatment with a 2-wk washout (Figure 1). During the whole study, all subjects were asked to follow a low polyphenol diet (LPD) that excluded berries, apples, pears, citrus fruits, fruit juices, onions, gynura, basil, bok choy, spinach, rabbit milkweed, brassica napus, chocolate, wine, coffee, tea, beans, nuts, soy related products, and most spices. Following the run-in phase, 16 volunteers were assigned randomly to either the PSPL or LPD diet (n = 8). Lunch and dinner meals were provided to all subjects during the whole study. The duration of the whole study was 7 wks, including 1-wk run-in and 2 phases of 2-wk dietary treatment stored at -20°C for determinations of total phenolic content and 8-OHdG.

Sample collection and storage

Six fasted venous blood samples were collected from each subject between 7-9 AM in the study (Fig. 1). Following centrifugation at 1000 x g for 10 min at 4°C, aliquots of plasma samples were snap frozen in liquid nitrogen and stored at -80°C. One aliquot of fresh plasma was used immediately for the LDL oxidation assay on the same day. After washed with ice-cooled saline three times and hemolyzed using ice-cooled distilled water, erythrocytes were stored at -80°C for glutathione (GSH) determination. A total of six 24-h urine samples were collected from each subject on the same day of blood collection. Urine was collected into an amber plastic container and stored at 4°C before it was brought back to the lab. After the volume was recorded, aliquots of urine samples were stored at -20°C for determinations of total phenolic content and 8-OHdG.

Biomarkers of antioxidant defense and oxidative stress

Total phenolic contents in urine and plasma were measured using the Folin-Ciocalteau’s reaction, according to the method of Singleton. Results were expressed as gallic acid equivalents (GAE) µmol/L.

Plasma α-Tocopherol was measured using a HPLC method of Milne and Botnen. Total antioxidant status (TAS) in plasma was assessed using a commercial enzymatic assay (Randox, UK). Reduced GSH in erythrocytes was determined using a commercial enzymatic assay (Calbiochem Co., CA, USA). Plasma malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE), products of lipid peroxidation, were measured using a commercial enzymatic assay (Calbiochem Co., USA). Urinary 8-OHdG was determined using an ELISA assay (Japan Institute for the Control of Aging, Japan). The resistance of LDL against Cu²⁺-induced oxidation was determined according to the slightly modified method of Chen et al. Briefly, following a 24-hour dialysis against saline containing Na-EDTA (1 mmol/L), LDL protein was quantified using a Bio-Rad protein assay kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Subsequently, LDL (182 mmol/L) was oxidized by 10 µmol/L CuSO₄ in a final volume of 1.0 mL. Formation of conjugated dienes was

Figure 1. Study design
Antioxidant activity of purple sweet potato leaves monitored by absorbance at 234 nm at 37°C over 6 hour using a UV3000 spectrophotometer (Hitachi, Japan) equipped with a 6-position automated sample changer. The results of the LDL oxidation are expressed as lag time (defined as the intercept at the abscissa in the diene-time plot).

**Statistical analysis**

All results were reported as mean ± SD. Repeat ANOVA (mix-model) analysis was performed to evaluate changes in parameters in the same dietary group over three time points, student’s t test was performed to evaluate changes from the d 0 value (D7-D0 and D14-D0) between LPD and PSPL dietary group. p value ≤0.05 was considered significant. The SAS statistical software package (SAS Institute Inc., Cary, NC) was used to perform all statistical analyses.

**RESULTS**

During the study period, a balanced diet provided to the subjects contained 2000 ± 200 Kcal, 95 ± 10 g protein, 250 ± 25 g carbohydrate, and 69 ± 7 g fat. 200 g cooked PSPL contained 60 Kcal, 6.6 g protein, 1.2 g fat, 9.2 g carbohydrate, 38 mg vitamin C, 170 mg Ca, 40 mg Mg, 902 mg total phenols, and 47.5 mg carotenoids. All 16 subjects completed the 7-wk study and were fully compliant to the LPD, based on the results of dietary records. No significant changes in their BMI, total body fat and clinical biochemistries were observed (Table 1).

PSPL addition to the LPD maintained total phenolic content in plasma while the LPD alone led to a significant 3.3% decrease from 3.59 ± 0.11 to 3.47 ± 0.08 µmol/L at d 14 as compared to that at d 0 (p ≤0.05). Similarly, urinary total phenolic excretion in the LPD group was decreased significantly by 16.3% from 0.49 ± 0.07 µmol/L at d 0 to 0.41 ± 0.08 µmol/L at d 14 (Figure 2). However, urinary total phenolic excretion in the PSPL group was significantly augmented by 24.5% at d 14 as compared to that at d 0 (p ≤0.05). Further, the increased plasma total phenolic content from d 14 to d 0 in the PSPL was significantly different from the slightly decreased value in the LPD (p ≤0.05). Similarly, increases in urinary phenolics in the PSPL group at day 7 and 14 as compared day 0 were significantly different from those in the LPD group.

At day 14, PSPL and LPD both decreased plasma α-tocopherol by 31.7 and 15.8% as compared to day 0, respectively (Table 2). Further, the decrease was larger in subjects consuming PSPL than LPD. Erythrocyte GSH status was not significantly altered by the LPD from day 0 to 14, while its concentration was enhanced significantly by PSPL consumption by 33.3 % at day 14 vs. day 0. Further, the increase in erythrocyte GSH from day 0 to day 14 was significantly 72% larger as a result of PSPL intake than the LPD. Total antioxidant status was not significantly altered by the LPD and PSPL from day 0 to 14.

Plasma concentrations of MDA+HNE in the subjects consuming the LPD were significantly decreased by 4.0% after 1 wk (p ≤0.05). The addition of PSPL into the LPD led to a significant decrease in MDA+HNE by 6.4 % and 5.1% at day 7 and day 14 as compared to that at day 0. However, MDA+HNE concentration at day 14 in the

| Table 1. Demographic characteristics and clinical biochemistries of subjects |
|----------------|----------------|
|                | Before         | After          |
| Age (yr)       | 20.4±1.8       | 21.1±2.2       |
| Height (cm)    | 167.8±9.1      | 167.8±9.1      |
| Body weight (kg)| 58.7±9.2      | 59.6±9.2       |
| BMI (kg/m2)    | 20.8±2.3       | 21.1±2.2       |
| Body fat (%)   | 22.3±6.8       | 23.8±7.0       |
| Creatinine (mg/dL) | 0.89±0.15  | 0.92±0.15     |
| GOT (IU/L)     | 19.7±6.16      | 19.0±4.40      |
| GPT (IU/L)     | 14.80±8.58     | 13.7±5.94      |
| Triglyceride (mg/dL) | 62.2±21.3 | 73.4±55.8     |
| Cholesterol (mg/dL) | 156±24.9    | 156±24.9      |
| HDL-Cholesterol (mg/dL) | 58.3±15.8 | 58.6±17.9     |
| LDL-Cholesterol (mg/dL) | 86.1±17.9 | 82.3±20.2     |

1Results were expressed as mean ± SD(n = 16).

**Means significantly differ, tested using pair-t test (p ≤0.05).**
Table 2. The status of antioxidants in subjects†

<table>
<thead>
<tr>
<th></th>
<th>D0</th>
<th>D7</th>
<th>D14</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma α-tocopherol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(μmol/L)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>LPD</td>
<td>6.2 ± 0.8</td>
<td>7.3 ± 0.8a</td>
<td>5.2 ± 0.6ab</td>
</tr>
<tr>
<td>PSPL</td>
<td>11.4 ± 1.4</td>
<td>9.8 ± 1.4a</td>
<td>7.8 ± 0.8ab</td>
</tr>
<tr>
<td><strong>Erythrocyte GSH</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(μmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPD</td>
<td>18.5 ± 7.9</td>
<td>18.2 ± 7.0</td>
<td>23.5 ± 6.6</td>
</tr>
<tr>
<td>PSPL</td>
<td>25.9 ± 11.7</td>
<td>25.4 ± 10.4</td>
<td>34.5 ± 7.4a</td>
</tr>
<tr>
<td><strong>Plasma total antioxidant status</strong> (mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPD</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>PSPL</td>
<td>0.8 ± 0.1</td>
<td>0.9 ± 0.2</td>
<td>0.9 ± 0.2</td>
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</tbody>
</table>

†Results were expressed as mean ± SD (n = 16).
a Means significantly differ as compared with D0, ab Means significantly differ between D7 and D14, tested using mix model analysis (p ≤ 0.05).

Table 3. The status of oxidative stress†

<table>
<thead>
<tr>
<th></th>
<th>D0</th>
<th>D7</th>
<th>D14</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MDA+4HNE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(μmol/L)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>LPD</td>
<td>7.5 ± 0.1</td>
<td>7.2 ± 0.2a</td>
<td>7.4 ± 0.3b</td>
</tr>
<tr>
<td>PSPL</td>
<td>7.8 ± 0.3</td>
<td>7.3 ± 0.3a</td>
<td>7.4 ± 0.2a</td>
</tr>
<tr>
<td><strong>8-OHdG</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(ng/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPD</td>
<td>10.2 ± 5.8</td>
<td>9.9 ± 4.8</td>
<td>8.5 ± 3.7</td>
</tr>
<tr>
<td>PSPL</td>
<td>8.1 ± 5.6</td>
<td>5.1 ± 4.5a</td>
<td>6.9 ± 3.1</td>
</tr>
<tr>
<td><strong>LDL lag time</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPD</td>
<td>73.6 ± 13.9</td>
<td>74.7 ± 10.0</td>
<td>78.1 ± 14.0</td>
</tr>
<tr>
<td>PSPL</td>
<td>78.0 ± 12.9</td>
<td>87.1 ± 31.0</td>
<td>89.7 ± 16.5a</td>
</tr>
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</table>

†Results were expressed as mean ± SD (n = 16).
a Means significantly differ as compared with D0, a Means significantly differ between D7 and D14, tested using mix model analysis (p ≤ 0.05).

DISCUSSION

Polyphenols in plant foods may contribute to decreased risk of chronic diseases because of an array of their putative mechanism of actions, i.e., antioxidation, anti-inflammation, and anti-proliferation. Purple sweet potato leaves have been commonly consumed in Asian countries. Since they are rich in various nutrients, incorporation of PSPL into the daily diet may provide benefits in health promotion and prevention. In this study, we observed that the incorporation of 200 g/d PSPL into the LPD for 2 wks enhanced antioxidant defense and decreased oxidative stress in healthy subjects.

Since polyphenols are ubiquitous in plant foods, they are an integral part of our daily diets. It has been estimated that average polyphenol intake probably reaches 1 g/d in people who eat several serving of fruit and vegetables per day. In this study, 902 mg polyphenols from 200 g PSPL added to the LPD provided a comparable quantity of polyphenol intake to the reported value. Consistent with no adverse effect reported in human studies, there were no apparent adverse effects after the consumption of 200 g/d PSPL for 2 wks, according to results of unaltered values of clinical biochemistries, serum creatinin, glutamin oxaloacetate transaminase (GOT), glutamic pyruvic acid transaminase (GPT), triglyceride, and cholesterol, as well as no reported gastrointestinal discomforts (diarrhea, abdominal pain or bloating).

Bioavailability of polyphenols has been documented commonly in humans. Our results showed that 902 mg of polyphenols derived from 200 g/d PSPL consumption enhanced plasma total phenolic content and urinary phenolic excretion after consuming PSPL for 2 wk might be a result of a rapid clearance of polyphenols because half-lives of polyphenolic compounds are generally shorter than 12 h.

Reactive oxidant species are believed to play an etiological role in pathogenesis of chronic diseases and aging. Well documented antioxidant actions of polyphenols may partially account for decreased risk of oxidative stress-related chronic diseases. Because subjects in this study are healthy and may experience a low degree of oxidative stress, we did not observe the impact of PSPL polyphenols on plasma α-tocopherol status, an outcome consistent to results of our rat study that flavonol quercetin could not prevent decreases in plasma and tissue α-tocopherol in rats fed a PSPL diet. On the other hand, a relatively smaller increase in plasma total phenolic content than changes in urinary phenolic excretion after consuming PSPL for 2 wk might be a result of a rapid clearance of polyphenols because half-lives of polyphenolic compounds are generally shorter than 12 h.

PSPL group was not different from those at day 7. Urinary 8-OHdG, a systematic biomarker of DNA damage, was employed to reveal antioxidant action of constituents in PSPL (Table 3). The LPD did not alter urinary 8-OHdG value from day 0 to day 14 while PSPL consumption significantly decreased urinary 8-OHdG by 3.67% at day 7 as compared to that at day 0 (p ≤ 0.05). Further, the decrease in 8-OHdG by PSPL consumption from day 0 to day 7 was significantly larger than that by the LPD. The resistance of LDL against Cu2+-induced oxidation significantly increased by 15% after consumption of PSPL for 2 weeks while no significant changes were found in the LPD group. (Table 3).
phenols didn’t enhance plasma total antioxidant status. The direct radical quenching activity of polyphenols in vivo has been questioned because of their relatively low circulating concentrations as compared to plasma uric and ascorbic acid. In addition to being annihilated by antioxidant defense, escaped reactive oxidant species can attack macromolecules and thereby cause pathogenesis of some diseases. For example, radical-mediated DNA damages are associated with carcinogenesis and oxidized LDL involve in atherogenesis. A growing body of evidence from in vitro, preclinical, and clinical studies suggested that polyphenols including flavonoids could protect LDL, DNA, protein, and lipid against oxidation. In this study, neither LPD nor 200 g/d PSPL altered the magnitudes of in vivo lipid peroxidation in apparently healthy individuals. In this study, the TBARS assay employed to assess MDA+4-HNE might be inadequate to reveal magnitude of in vivo lipid peroxidation because of appreciated interferences from bilirubin, sugar, and other factors. Interestingly, PSPL antioxidants decreased urinary excretion of DNA oxidation products temporarily in subjects in the PSPL group after the first week, but not after the second week. Similarly, polyphenols in onions and green tea diminished urinary 8-OHdG excretion in humans. Al- though these results of decreased urinary 8-OHdG excretion could be interpreted as a decrease in oxidant-induced DNA damage via antioxidative actions of polyphenols, it might simply suggest decreased capacities of DNA repairing mechanisms. In contrast to evidence from in vitro studies indicating antioxidative activity of polyphenols, our results suggested that antioxidative actions of PSPL polyphenols or other constituents might not be effective to diminish magnitudes of DNA and lipid oxidation in young healthy individuals when their endogenous anti- oxidant defense system is adequate to minimize in vivo oxidant-induced damages. In vitro studies revealed, that polyphenols act as an oxygen radical scavenger as they enhance the resistance of LDL against Cu2+-induced oxidation. Our study showed significantly increased LDL lag time after PSPL consumption for 2 weeks. Further, antioxidative actions of PSPL polyphenols that might be too subtle to be detected in a Cu2+-induced ex vivo oxidation model could be unmasked with in vitro addition of antioxidants. The interactive effects among PSPL constituents, such as polyphenols and carotenoids, on antioxidant defense and oxidative stress remain to be investigated.

In conclusion, 902 mg of polyphenols in 200 g/d PSPL could be bioavailable and enhance glutathione status and decrease LDL oxidation. However, their antioxidative actions might not be sufficiently potent to modulate overall antioxidant defense in young healthy individuals.

ACKNOWLEDGEMENT
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AUTHOR DISCLOSURES

REFERENCES


Original Article

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攝取紅甘藷葉飲食可以降低健康成年人體內的脂質過氧化作用及 DNA 的損傷

攝取富含多酚類的食物，與減少慢性疾病的風險有關，其可能的生化機制包括增強抗氧化及抗發炎等。紅甘藷葉（Purple sweet potato leaves, PSPL）富含豐富的多酚類，是亞洲人經常食用的蔬菜。在這個交叉試驗中，提供 16 位健康受試者（年齡 20-22 歲，7 男 9 女），每日 200 公克紅甘藷葉（包含 902 毫克總多酚類），以探討其對人體抗氧化的影響。於一星期的適應期之後，將所有受試者隨機分成 2 組，實驗組每日攝取 200 公克紅甘藷葉，對照組則攝取低多酚類飲食，持續 2 週之後再進行 2 星期的排空期，之後 2 組飲食對調，再進行持續 2 週的試驗。分別收集受試者於每個試驗期的第 0、7、14 天的空腹血液及 24 小時尿液進行分析。結果顯示，攝取紅甘藷葉 14 天後，明顯增加受試者尿中多酚類的排泄，比第 0 天增加了 24.5%；但攝取低多酚類飲食 2 週之後，受試者的血漿及尿液中總多酚類的含量明顯減少，分別為 3.3 % 及 16.3 %。攝取紅甘藷葉 14 天後，低密度脂蛋白氧化遲滯時間（Low-density lipoprotein lag time）及紅血球中 glutathione 的濃度，分別比第 0 天時明顯提升了 15% 及 33.3%；而攝取低多酚類飲食組則沒有明顯變化。尿中 8-hydroxy-deoxyguanosine (8-OHdG) 的排泄方面，在攝取紅甘藷葉 7 天後，明顯比第 0 天減少了 36.7%；而攝取低多酚類飲食組則沒有明顯變化。綜合以上的結果，200 公克紅甘藷葉中的多酚類是可被人體吸收利用的，且可以降低體內脂質過氧化作用、DNA 的損傷及增加抗氧化防禦能力，以減少健康年輕人的氧化壓力。

關鍵字: 紅甘藷葉、多酚類、脂質過氧化作用、DNA 損傷