Regulatory/modulatory effect of prune essence concentrate on intestinal function and blood lipids

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ABSTRACT

Context: Prunus domestica Linn (Rosaceae) has been considered a functional food, owing to its various pharmacological activities, including antioxidant, anti-inflammatory, antidiabetic and anticancer.

Objective: This placebo-controlled, randomized study was framed to check the beneficial activity of prune essence concentrates (PEC) in corroboration with intestinal function and lipid profile in mildly hypercholesterolemic subjects.

Materials and methods: Sixty healthy mild hypercholesterolemic subjects were randomly chosen and segregated into three groups as placebo (consume 50 mL of simulated prune drink), PEC I (consume 50 mL of PEC/day) and PEC II (consume 100 mL of PEC/day) for 4 weeks with 2 weeks of follow-up without PEC consumption.

Results: Intake of PEC (I and II) for 4 weeks substantially ameliorated (p < 0.05) the colony number of Clostridium perfringens (5.97 and 8.35%) and Escherichia coli (6.25 and 9.38%). Meanwhile, the total cholesterol (TC; 5.90 and 6.99%) levels and LDL-c (6.68 and 6.53%) were significantly reduced (p < 0.05), but no change in other lipid parameters. Whereas, the antioxidant capacity was also concomitantly elevated (p < 0.05) upon administration with PEC.

Discussion and conclusion: Overall, the results suggest that the use of PEC may positively regulate the intestinal microflora and thereby effectively lower the TC levels and thus act as a hypocholesterolemic agent.

Introduction

Epidemiological studies have indicated that cardiovascular diseases (CVDs) claim more lives than any other diseases. In Taiwan, CVDs are the second largest contributor to mortality, which accounts for almost 11.4% of all mortality (Teo et al. 2013; Chiu et al. 2015). Hypercholesterolemia is a condition in which serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-c) are substantially elevated and thus believed as a dominant risk factor in the pathophysiology of atherosclerosis and subsequent CVDs (Lu et al. 2015). Several researchers highlighted that maintenance of lipid levels in normal level (normcholesterolemia) might reduce the incidence of CVDs. Currently several lipid-lowering drugs are available in the markets, however, they cause several adverse effects, which leads to the quest for functional food/nutraceuticals with hypocholesterolemic efficacy without triggering any adverse effect is increased enormously.

Experimental data suggested that increased intake of fruits and vegetables may cut down the onset of CVDs, due to the presence of photo components (polyphenols; flavonoids), which attributes for its antioxidant and anti-inflammatory properties (Slavin & Lloyd 2012; Chiu et al. 2015). Especially, fruits (juice or dried form) like apple, berries, raisins, apricot, dates, and prunes are reported to lower the incidence of CVDs (Oyebode et al. 2014). Prune is a dried form of plum fruits of Prunus domestica Linn (Rosaceae) Prune is usually processed as juice/essence, jam, puree or other prune products. Several studies demonstrated that prune is rich in both soluble and insoluble fibers, simple sugars especially xylo-oligosaccharides, phenolic acid derivatives (chlorogenic acid, neochlorogenic acid, cryptochlorogenic acid, and oligomeric proanthocyanidin) as well as micronutrients like vitamins and minerals (Stacewicz-Sapuntzakis et al. 2001; Putnam et al. 2007). Prunes are reported to exhibit numerous therapeutic properties including antioxidant, antidiabetic, anticancer, as well as cardioprotective, gastroprotective (laxative) and neuroprotective agents. In addition, it is also recommended for treating bone mineral loss and menstrual related disorders (Stacewicz-Sapuntzakis 2013; Igwe & Charlton 2016).

Existing evidence suggests that fruits rich in fibers and oligosaccharides might act as prebiotics (feeds probiotics) and thus promote the growth of the healthy microorganism (intestinal...
microflora) and thereby enhance various physiological functions (El-Gawad et al. 2005; Scheid et al. 2013). An impressive number of studies indicated that intestinal microflora plays a pivotal role in maintaining human health status by ameliorating nutrient absorption, effectively removing toxins, maintaining normal mucosal immunity, suppressing pathogen colonization and regulating fat metabolism (Alonso & Guarner 2013; Qiao et al. 2014). Prune has been demonstrated to possess several biological activities (especially hypcholesterolemic). However, the link between prune intake with intestinal microbiota and lipid status as well as its association were not conducted until now. Hence, the current trial was carried out to assess whether the consumption of prune essence concentrates (PEC) modulate the intestinal microflora, antioxidant status and lipid profile in mildly hypercholesterolemic subjects.

Materials and methods

Commercial PEC and placebo

Both PEC and placebo were provided by Cerebos Pacific Ltd., Singapore, as BRAND InnerShine Prune Essence. The major ingredients of PEC (each bottle) were summarized as follows: 6 g of dietary fiber (pectin), 0.8 g of oligosaccharides (inulin, xylo-oligosaccharides), 1 g of fructose and trace of sorbitol, malic acid, sodium citrate with water (42.1 kcal/bottle). The placebo beverage contained brown sugar, pectin, malic acid, sodium citrate and simulated with prune-washed water. Both the sample bottles looked alike with similar colour, appearance, flavour, size and shape.

Subjects and study design

Sixty healthy mild hypercholesterolemic subjects (aged between 18 and 53 years; 27 males and 33 females) were recruited by flyers and advertisement posted in public places and Chung Shan Medical University Hospital, Taiwan. The volunteers included in the present randomized, placebo-controlled study based on medical history questionnaires along with dietary habits and vitals were checked to confirm the health status. The main inclusion criteria included mild hypercholesterolemia (serum cholesterol 170–200 mg/dL) without any metabolic disorder, and the exclusion criteria included the history of drinking, smoking, pregnancy, breastfeeding, chronic illness, gastrointestinal disorders, uncontrolled diabetes mellitus, cardiac or renal dysfunction as well as intake of dietary or nutritive supplements and or any other medications. Volunteers were requested to sign a consent form prior to the intervention.

The current study was approved by the Institutional Review Board (IRB) of the Chung Shan Medical University Hospital, Taichung, Taiwan and conducted (CSMUH: CS10056) in accordance with the guidelines laid down in the Declaration of Helsinki and Good Clinical Practice. All the sixty healthy mild hypercholesterolemic subjects were randomly divided (by digital computerized codes) into three groups as placebo (n = 20), who were instructed to consume one bottle (50 mL) of simalted drink with prune-washed water, PEC I (test A; n = 20), who were requested to consume one bottle (50 mL) of PEC/day (after a meal) and PEC II (test B; n = 20), who were requested to consume 2 bottles (100 mL) of PEC/day for 4 weeks. The total experimental period was carried out for seven weeks, out of which first week (run-in period) was for adaptation or stabilization, followed by four weeks as an intervention period (consumption of samples) and lastly two weeks as follow-up period without any consumption of samples. During the intervention, subjects were requested to abide with normal lifestyle and dietary pattern. Anthropometric measurements were done along with collections of both faecal and blood samples. Based on subject records, the average consumption rate of PEC was about 90% at the end of the experiment. During the intervention period, one female subject in the placebo group, as well as one male from PEC (test A) group was excluded because of personal reasons, and hence only 58 subjects completed the study.

Blood collection

The fasting blood samples (initial, 4th and 6th weeks) were collected in two tubes, one with EDTA coated for plasma and another without anticoagulant for serum preparation. Plasma was separated and used for evaluating various antioxidant indexes. The serum samples were used for determining the lipid profile. All the blood samples were stored at −80°C until analysis.

Faecal sample collection and bacterial enumeration

Faecal samples were collected and weighed, then transferred into the sterile plastic anaerobic bag at initial, 2nd, 4th and 6th week to check the major intestinal microflora. One gram of faecal sample was mixed with 99 mL of peptone saline (peptone and sodium chloride) and homogenized (10%) in different dilution ratios with peptone saline in an anaerobic condition. To quantify aerobic and anaerobic microflora, 20 μL of the homogenized faecal sample was plated on acidified different agar medium and incubated for 48 h in aerobic and anaerobic conditions at 37°C based on different types of bacterial enumeration. Enumeration of Escherichia coli, Clostridium perfringens, Lactobacillus spp., Bifidobacteria spp. and total anaerobic bacteria were done on MacConkey agar (Merck, Germany) by the method of Manafi and Kneifel (1989), TSC agar by Harmon et al. (1971), Rogosa SL agar (Merck, Germany) by Rogosa et al. (1951), BIM 25 agar and Brucella agar (Creative Microbiologicals, Taiwan) by Beerens (1991), respectively. After the incubation period, colonies were identified and characterized by Gram staining, cellular morphology, and catalase reaction. The colonies of various bacterial spp. were calculated by using a formula with dilution factor and dry weight of faeces. The colony counts were reported as log10 of colony forming units (CFU/g dry weight of faeces).

Lipid profiles in serum and oxidative indexes in plasma

The total cholesterol (TC), triglyceride (TG) and high-density lipoprotein cholesterol (HDL-c) were measured by commercial lipid profile kits from Roche Diagnostics (Mannheim, Germany). Low-density lipoprotein cholesterol (LDL-c) was calculated using the Friedewald equation. Total antioxidant capacity in plasma was determined by the method of Miller et al. (1993) with a slight alteration. The total TBARS in plasma was measured by reacting with 2-thiobarbituric acid using the Draper and Hadley (1990) method.

Statistical analysis

The outcomes were expressed as a mean± standard deviation (SD). The paired t-test was used to compare the changes in the same group, and Student’s t-test was used to compare the
difference between the PEC and placebo groups and the variables were analyzed via one-way ANOVA with a post hoc LSD test using Statistical Package for the Social Sciences (SPSS) version 23.0 (IBM, Chicago, IL). p-values <0.05 are deemed as statistically significant.

**Results**

The effect of PEC on anthropometric parameters in healthy mild hypercholesterolemic subjects is summarized in Table 1. No substantial alterations were observed in any of the levels of anthropometric parameters, such as body weight, BMI and body fat in test (PEC I and II) or placebo-ingested subjects. However, a slight decrease in BMI and body weight was noted in both test groups in the follow-up period, but not significant. The schematic representation of the present study is portrayed in Figure 1.

Table 2 exemplifies the effect of PEC on the intestinal microflora in healthy mild hypercholesterolemic subjects. Four weeks of treatment with PEC (I and II) exhibited a selective escalation in the intestinal beneficial bacterial population, especially *Bifidobacterium*, *Lactobacillus* spp. and total anaerobic bacterial count on equivalence with the initial value. In comparison with placebo group the count of *Bifidobacterium*, *Lactobacillus* spp. and the total anaerobic bacterial count was substantially elevated at the end of the intervention (4th week). Meanwhile, the harmful bacteria like *C. perfringens* and *E. coli* were greatly restrained upon treatment with PEC. However, during the follow-up (6th week) *C. perfringens* and *E. coli* were slightly increased, whereas *Bifidobacterium*, *Lactobacillus* spp. and the total anaerobic bacterial count was markedly reduced due to stoppage of PEC treatment.

Table 3 represents the effect of PEC on lipid profile in healthy mild hypercholesterolemic subjects. PEC (I and II) ingested subjects showed a notable decrease (p < 0.05) in the levels of TC, LDL-c with a minor improvement in the levels of HDL-c as compared with baseline (0 week). After 4 weeks of intervention, the levels of both TC and LDL-c in PEC I and II were concomitantly decreased in comparison with the placebo group. Nevertheless, during the follow-up period, the levels of TC and LDL-c were mildly elevated (Table 2). In the case of TG in any of the group did not infer any changes.

The efficacy of PEC on the oxidative indexes in healthy mild hypercholesterolemic subjects is summarized in Table 4. In equivalence with placebo, PEC-consumed subjects showed a considerable augmentation in the levels of TEAC with a substantial alleviation (p < 0.05) in TBARS levels. Four weeks of treatment with PEC I and II showed considerable changes in the concentration of TBARS and TEAC compared with the baseline. There were no substantial changes observed in the placebo group for both TEAC and TBARS. Overall, both the PEC groups showed notable changes on equivalence with placebo. However, PEC II

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**Table 1.** The effect of PEC on anthropometric parameters in healthy mild hypercholesterolemic subjects.

| Weeks     | Groups   | Weight (kg) | BMI (kg/m²) | Body fat (%) |
|-----------|----------|-------------|-------------|--------------|
| Initial   | Placebo  | 64.29 ± 13.21a | 23.59 ± 4.04a | 25.99 ± 5.80a |
|           | PEC I    | 59.88 ± 11.03a | 21.57 ± 2.45a | 24.25 ± 4.14a |
|           | PEC II   | 63.50 ± 12.60a | 22.82 ± 2.98a | 25.99 ± 4.64a |
| 4th Week  | Placebo  | 63.99 ± 13.66a | 23.48 ± 4.24a | 25.16 ± 4.00a |
|           | PEC I    | 59.65 ± 10.75a | 21.49 ± 2.33a | 24.39 ± 4.85a |
|           | PEC II   | 63.60 ± 12.70a | 23.02 ± 2.97a | 25.67 ± 4.66a |
| Follow-up (6th week) | Placebo  | 63.89 ± 11.34a | 23.62 ± 4.08a | 25.56 ± 5.31a |
|           | PEC I    | 60.01 ± 11.86a | 21.25 ± 2.48a | 24.17 ± 4.22a |
|           | PEC II   | 63.31 ± 12.50a | 22.90 ± 2.68a | 25.93 ± 4.73a |

Values are expressed as means ± SD. Data within the same group bearing different superscripts letters were significantly different (p < 0.05). BMI: Body mass index.

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**Figure 1.** The schematic representation of the present study.
Table 2. The effect of PEC on the intestinal microflora count in healthy mild hypercholesterolemic subjects.

| Weeks | Groups | C. perfringes (CFU/g) | Lactobacillus spp (CFU/g) | Bifidobacterium spp (CFU/g) | TAB (CFU/g) |
|-------|--------|-----------------------|--------------------------|----------------------------|-------------|
| Initial | Placebo | 7.99 ± 0.43a | 8.03 ± 0.31a | 8.24 ± 0.93a | 7.99 ± 0.66a | 9.60 ± 0.32a |
|        | PEC I  | 7.99 ± 0.28a | 8.03 ± 0.43a | 8.09 ± 0.51c | 7.06 ± 0.53c | 9.36 ± 0.30c |
|        | PEC II | 7.67 ± 0.20a | 8.02 ± 0.44a | 8.09 ± 0.69c | 7.52 ± 0.64c | 9.25 ± 0.70c |
| 2nd Week | Placebo | 7.77 ± 0.48a | 8.03 ± 0.30a | 8.33 ± 0.52a | 8.02 ± 0.72a | 9.66 ± 0.41a |
|        | PEC I  | 7.75 ± 0.30a | 7.99 ± 0.42a | 8.24 ± 1.39b | 7.94 ± 0.59b | 9.65 ± 0.29g |
|        | PEC II | 7.28 ± 0.25h, | 7.67 ± 0.42h, | 8.93 ± 0.66h, | 8.61 ± 0.61h, | 9.75 ± 0.93h |
| 4th Week | Placebo | 7.80 ± 0.43a | 8.02 ± 0.31a | 8.25 ± 0.97a | 8.07 ± 0.86a | 9.64 ± 0.42a |
|        | PEC I  | 7.49 ± 0.27h, | 7.55 ± 0.41i, | 8.66 ± 0.86h, | 8.37 ± 0.65s | 9.95 ± 0.34a |
|        | PEC II | 6.95 ± 0.30i, | 7.35 ± 0.38i, | 9.41 ± 0.48i, | 8.98 ± 0.33i, | 10.21 ± 0.43i |
| Follow-up (6th week) | Placebo | 7.77 ± 0.43a | 8.21 ± 0.29a | 8.46 ± 0.56c | 8.00 ± 0.87r | 9.68 ± 0.50a |
|        | PEC I  | 7.93 ± 0.24a | 8.17 ± 0.46a | 8.02 ± 0.42c | 7.37 ± 0.43bc | 9.30 ± 0.32b |
|        | PEC II | 7.54 ± 0.23a | 8.16 ± 0.38a | 8.39 ± 0.41b | 7.95 ± 0.50r | 9.39 ± 0.19b |

Values are expressed as means ± SD. Data within the same group bearing different superscripts letters were significantly different (p < 0.05).

* p < 0.05,
** p < 0.01 (Placebo vs PEC I and II in 2nd and 4th week). TAB: Total anaerobic bacteria.

Table 3. The effect of PEC on the plasma lipid profile in healthy mild hypercholesterolemic subjects.

| Weeks | Groups | TG (mg/dL) | TC (mg/dL) | LDL (mg/dL) | HDL (mg/dL) |
|-------|--------|------------|------------|-------------|-------------|
| Initial | Placebo | 82.60 ± 16.31a | 179.10 ± 27.43a | 99.30 ± 20.20a | 58.95 ± 8.06a |
|        | PEC I  | 87.20 ± 13.67a | 173.45 ± 24.83a | 98.80 ± 26.10a | 63.70 ± 8.66a |
|        | PEC II | 85.45 ± 23.93a | 179.40 ± 31.47a | 104.10 ± 30.36a | 58.80 ± 9.97a |
| 4th Week | Placebo | 86.80 ± 18.40a | 174.70 ± 22.59a | 98.46 ± 18.36a | 57.30 ± 7.86a |
|        | PEC I  | 79.60 ± 15.12a | 163.20 ± 28.24h, | 92.20 ± 25.67p, | 65.65 ± 8.88 |
|        | PEC II | 88.21 ± 21.34a | 166.85 ± 28.45h, | 98.34 ± 27.50p, | 59.70 ± 10.67 |
| Follow-up (6th week) | Placebo | 85.01 ± 21.04a | 176.23 ± 24.45a | 99.23 ± 19.41a | 57.05 ± 9.05a |
|        | PEC I  | 78.45 ± 14.94a | 169.29 ± 29.20a | 96.45 ± 22.56a | 64.70 ± 6.01a |
|        | PEC II | 87.35 ± 20.25a | 171.67 ± 30.63a | 103.73 ± 21.82a | 60.10 ± 9.66a |

Values are expressed as means ± SD. Data within the same group bearing different superscripts letters were significantly different (p < 0.05).

* p < 0.05,
** p < 0.01 (Placebo vs PEC I and II in 4th week).

Table 4. The effect of FPE on the oxidative indexes in healthy mild hypercholesterolemic subjects.

| Weeks | Groups | TEAC (mM) | TBARS (µM) |
|-------|--------|-----------|-----------|
| Initial | Placebo | 0.41 ± 0.02a | 0.82 ± 0.14a |
|        | PEC I  | 0.40 ± 0.05a | 0.82 ± 0.21a |
|        | PEC II | 0.40 ± 0.07a | 0.81 ± 0.18a |
| 4th Week | Placebo | 0.41 ± 0.06a | 0.83 ± 0.11a |
|        | PEC I  | 0.43 ± 0.05a | 0.80 ± 0.13a |
|        | PEC II | 0.43 ± 0.06a | 0.69 ± 0.07a |
| Follow-up (6th week) | Placebo | 0.42 ± 0.03a | 0.81 ± 0.09a |
|        | PEC I  | 0.41 ± 0.06a | 0.81 ± 0.08a |
|        | PEC II | 0.42 ± 0.05a | 0.74 ± 0.11a |

Values are expressed as means ± SD. Data within the same group bearing different superscripts letters were significantly different (p < 0.05).

* p < 0.05 (Placebo vs PEC I and II in 4th week).

showed much better results than PEC I, but no significant difference was noted among those groups.

**Discussion**

Several pre-clinical and clinical studies suggested that consumption of dried plum (prunes) extracts or concentrate suppressed the cholesterol level (hypercholesterolemic property) as well as lowered the LDL-oxidation, and thus attenuated the incidence of CVDs (Tinker et al. 1991; Stacewicz-Sapuntzakis et al. 2001; Stacewicz-Sapuntzakis 2013). Nevertheless, the exact association is yet to prove and also the clinical trial results are inconclusive. Hence, the present study was framed to assess whether consumption of PEC might alter the intestinal microflora and contributed to health-promoting activities like hypocholesterolemic and antioxidative activities. The HPLC fingerprinting data revealed the presence of various phenolic acids like chlorogenic acid, neochlorogenic acid and cryptochlorogenic acid (data not shown) and those beneficial effects of PEC might be due to those phenolic acids. Similarly, Putnam et al. (2007) demonstrated that chlorogenic and neochlorogenic acids are the crucial polyphenols which contribute to several biological functions in prune extract.

The anthropometric analysis was evaluated to confirm physiological changes related to body composition, after the administration of PEC. Intake of PEC I and II did not show any notable changes in the values of body weight, BMI and body fat. During the follow-up period (6th week), a minor decrement was observed in the levels of BMI and body weight, but the changes were not significant. The outcome of anthropometric analysis clearly demonstrated that ingestion of PEC for four weeks did not alter the body morphology or composition, and it was safe to consume prune concentrate without any adverse events. Likewise, the placebo group also did not showcase any changes in any of the anthropometric measurements.

The intestinal/gut microflora is a complex community of microorganisms (probiotics) such as bacteria, viruses, and protozoan, which reside in gastric tracts and feeds on undigested carbohydrates (prebiotics). The intestinal bacterial community is discriminated into beneficial and harmful bacteria (Fujimura et al. 2010). *Bifidobacterium* spp. and *Lactobacillus* spp. genera are classified as beneficial bacteria, since they render various health-promoting function by the production of short-chain fatty acids (SCFA) and vitamins, helping digestion and absorption of nutrients, immune-stimulant, inhibiting the growth of harmful bacteria or pathogens, lowering cholesterol and ammonia levels. Whereas, *Clostridium perfringes* spp. and *E. coli* are considered as harmful bacteria, which favour deleterious effects to human (Vendrame et al. 2011). PEC (I and II)-consumed subjects showed a remarkable improvement in the population of beneficial bacteria’s community, especially *Bifidobacterium*, *Lactobacillus* spp., and total.
The authors report that there is no conflict of interest to disclose.

Disclosure statement

The authors report that there is no conflict of interest to disclose.

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