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Hypocholesterolemic Effect of Spray Dried Pitaya Powder (SDPP) Among Normocholesterolemic Subjects in Mempaga, Bentong.

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Abstract. Red pitaya was reported to offer many health benefits including reduction in the mortality risk of cardiovascular disease. Studies showed that pitaya fruit is rich in vitamins, minerals and fibers that helps the digestive process, neutralize toxic substances such as heavy metals, and helps to reduce cholesterol levels and high blood pressure. The aim of this study was to evaluate the hypocholesterolemic effect of SDPP among normocholesterolemic subjects in Mempaga, Bentong. This study implied a clinical trial which was conducted among normocholesterolemic subjects. A total of 60 subjects were recruited from the residents of Felda Mempaga, Bentong, Pahang. In this single-blinded, cross-over supplementation trial, subjects were divided into 3 groups and were supplemented with 3 different dosages of SDPP. They were advised to consume normal diet as usual. For the first 4 weeks (W1-W4) of the intervention, subjects were given either 3, 4 or 5 sachets (20 gram each sachet) of SDPP to be consumed daily. The next 2 weeks (W5-W6) of the study was the wash-out period. After the wash-out period, starting from week seven to week 10 the treatment was crossed over among the subjects where those subjects that already consumed the products (SDPP) will become the control subjects (no supplementation). A medical doctor and a trained professional drawn a 15 ml fasting intervena blood sample for four times during the entire study period which was at the baseline week (W1), week 4, week 6 and week 10. The blood samples were analyzed for lipid profiles (TC, TG, HDL-C and LDL-C) using Hitachi Chemical Analyzer. Repeated Measures ANOVA analysis had shown a significant decrease ($p \leq 0.05$) in TC after 4 weeks supplementation of SDPP. All three dosages of SDPP showed a decreasing trend of TC. The range of TC dropped in SDPP group was 20.75% - 30.1% after 4 weeks supplementation. The higher the dosages given to the subjects, the higher the TC level dropped. Similarly, it was noted that SDPP supplementation revealed a significant decreased in LDL-C level ($p < 0.05$). LDL-C decreased by 21.88% (3 sachets SDPP), 25.89% (4 sachets SDPP) and 27.41% (5 sachets SDPP) respectively after 4 weeks of supplementation compared to baseline. Meanwhile, for TG and HDL-C levels, there was only a significant difference ($p < 0.05$) found in the group supplemented with 4 sachets of SDPP. In antioxidant status study, the consumption of 3 sachets of SDPP/ day did not alter the plasma TAS concentration. But, for both 4 sachets and 5 sachets of SDPP supplementation groups showed an increased percentage in TAS compared to baseline although only group with 5 sachets performed a significant increased ($p < 0.05$) by 12.31% after 4 weeks of consumption. These results could be predicted as a natural product containing high quantities of dietary fibers and antioxidant compounds positively influence plasma lipid levels and plasma antioxidant status in normocholesterolemic subjects.

Keywords: Red pitaya, Spray dried, Cross-over design, Total cholesterol

1. Introduction

Chronic non-communicable diseases are reaching epidemic proportions worldwide. Over the past years, cardiovascular disease (CVD) still ranked on top from the list. There are multiple risk factors contribute to CVD, which include cigarette smoking, elevated blood pressure, elevated LDL-cholesterol (LDL-C) and triglyceride levels, low HDL-cholesterol level (HDL-C), obesity and type 2 diabetes [1]. The emergence of these chronic diseases affects people of all ages, nationalities and classes, globally. From the statistic of year 2006 in United States, it had recorded that 50% from the breakdown of deaths due to cardiovascular disease

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were significantly attributed by heart disease and approximately it caused 1 of every 6 deaths [2]. Throughout the world, cardiovascular disease kills more people each year. This includes middle income country like Malaysia. In Malaysia, the prevalence of cardiovascular disease risk factors is high even in semirural community [3]. So far, the emphasis has been on the relationship between cholesterol levels and the risk of CHD. Studies have showed that elevated plasma total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) as the major risk factors for coronary heart disease [4]. Whereas, the protective effects against coronary heart disease are high concentrations of plasma high density lipoprotein cholesterol (HDL-C) and a low ratio of TC to HDL-C [5]. Suboptimal plasma levels of several micronutrients from fruits are inversely correlated with an increased risk of chronic degenerative diseases [6]. Within the population, the intake of fruit and vegetables varies according to a number of factors including sex, age, region and socioeconomic class. In Malaysia, the fruits and vegetables consumption was far below the recommendation by the WHO (400 gram or five servings) [7]. In a smaller scale screening programme also reported a high prevalence of respondents not consuming enough fruits and vegetables (92.1%) [8]. This probably due to the cost, the preparation of fruit and vegetables, often involving peeling, chopping and cooking, acts as a disincentive to eat these foods, in particular for people working outside the home. There is therefore considerable merit in looking for alternative convenient methods of administering the fruit and vegetables into products whilst retaining the same proportions of the active components as exists in the fresh foods. Pitaya or well known as dragon fruit, has attracted considerable consumer interest because of its micronutrient content and the vibrant color of the fruit itself. Dragon fruit is the fruit of several cactus species that have been classified as white (*Hylocereus undatus)*, red (*H. polyrhizus)*, and yellow (*Selenicereus megalanthus*) [9,10]. Red pitaya was reported to offer many health benefits including anti-inflammatory, chemoprevention of cancer, antidiabetic effects and a reduction in the mortality risk of cardiovascular disease [11,12]. This reports being supported by evidences that showed pitaya fruit is rich in vitamins, minerals and fibers that helps the digestive process, prevent colon cancer and diabetes, neutralize toxic substances such as heavy metals, and helps to reduce cholesterol levels and high blood pressure [13].

2. Materials and Methods

Representative samples of Red Pitaya Fruit (*Hylocereus polyrhizus*) were obtained from local plantation in Kluang, johor. The fruits were cleaned, washed, and the skin was peeled before being blended and homogenized for hydration process. The fruit juice was mixed with 23% Maltodextrin to avoid clogging in the spray dryer. After the juice had ready, the liquid in the pressure vessel is then pumped out and sent to the spray drier at a flow rate of 7 L/h to produce Spray Dried Pitaya powder. Then, mixtures were dried in a spray dryer at an inlet temperature of 180°C and an outlet temperature of 75°C. The SDPP produced was approximately 10% from the wet weight of the fresh fruit. The SDPP were packet into 20g/sachet for respondents' consumption. Table 1 showed the proximate composition of spray dried pitaya powder (SDPP).

Table 1: Proximate composition of spray dried red pitaya powder (g/100g dry weight)

Constituents	Value in SDPP
Moisture (%)	10.90 ± 1.6
Ash (g)	1.22 ± 0.49
Carbohydrate (g)	26.60 ± 5.88
Protein (g)	1.59 ± 0.25
Fat (g)	0.007 ± 0.007
Insoluble fiber (g)	12.05 ± 0.2
Soluble fiber (g)	5.65 ± 0.12
Total dietary fiber (g)	17.70 ± 0.16
Vitamin C (mg)	17.00 ± 0.12

Values were expressed as mean; each value is a mean of triplicate reading

This study was conducted at Mempaga, Bentong, Pahang, Malaysia. The majority of the respondents aged between 21 to 55 years old. 60 subjects were recruited for the study after conducting health screening.

Ethical approval for the study was obtained from the Ethical Committee, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, and all subjects gave signed informed consent prior to the participation in the study. Subjects were allowed to continue their normal dietary habits.

2.1. Subjects

Health screening was conducted prior to the experiment, and respondents who satisfied the following criteria were chosen to participate in the study:

- Volunteer
- Aged between 21 to 55 years old
- Normal total cholesterol level (< 5.2 mmol/l)
- Do not consume any medicine or supplements
- Free from disease

2.2. Study Design

The intervention trial study adapted the single-blinded, cross-over supplementation trial; with subjects were assigned at random to the treatment groups and control groups. The trial was a 10-weeks study which divided into 3 phases: intervention, wash-out period and again intervention. Firstly, a total of 120 subjects were screened and anthropometric and blood pressure measurements were taken. Blood glucose and total cholesterol level were obtained by a finger prick using Accucheck. Subjects that meet all inclusion criteria were eligible to participate in this study. These subjects were allocated into 3 different dosages. Subjects were consumed SDPP when they are in the treatment group. When they become control group, they will not consume any of them. They were advised to consume normal diet as usual. For the first 4 weeks (W1-W4) of the intervention, subjects were given either 3, 4 or 5 sachets (20 gram each sachet) of SDPP to be consumed daily. Subjects are required to record in their food record the daily time of the products consumption. The next 2 weeks (W5-W6) of the study is the wash-out period. After the wash-out period, starting from week seven to week 10 the treatment were crossed over among the subjects where those subjects that already consumed the products (SDPP) will become the control subjects (no supplementation).

2.3. Dietary and Physical Activity Analysis

Participants completed detailed three-day food and physical activity records. They were required to maintain the food and physical activity records for three (2) weekdays and one (1) weekend day. Prior to completing the food and physical activity records, all participants were provided with verbal and written instructions on maintaining the food records. Dietary intakes were analyzed using NutritionistPro software, version 4.1.0 (Axxa System, 4800 Sugar Grove Blvr. Suite 602 Stafford, Texas, USA). The average intake of three days was recorded.

2.4. Anthropometric Measurements

Weight was measured using a digital TANITA (+ 0.1 kg) scales and height was measured using SECA bodymeter (+0.1 cm).

2.5. Biochemical Analysis

A medical doctor and a trained professional drawn a 15 ml fasting intervena blood sample for four times during the entire study which is at the baseline week (W1), week 4 , week 6 and week 10. These blood samples were centrifuged at 3000rpm for 10 minutes at 4°C. Later, the blood samples were analyzed for glucose level, lipid profiles (TC, TG, HDL-C and LDL-C) and total antioxidant status (TAS) using the chemical auto analyzer machine (Hitachi, Roche Diagnostic, UK).

2.6. Statistical Analysis

Statistical calculations were performed using SPSS 15.0 software (SPSS Inc. Chicago, II, USA). Data was expressed as mean ± SEM. General linear model (repeated measures) were used to assess the effects of treatment for various parameters. In order to compare changes from the starting point of each groups paired t-test were used. Tukey's test was used for comparison of means. A value of $p < 0.05$ was used as a criterion for statistical significance.

3. Results and Discussion

A total of 60 subjects (26 males, 34 females) were selected from 120 participants to participate in this study. Table 2 represents the respondent sociodemographic background. All subjects were randomly selected and 20 subjects (n = 20) per group were allocated in each dosage (3 sachets, 4 sachets and 5 sachets) given.

3.1. Respondents Physical Characteristics

The mean body weight of the subjects was 67.3 ± 2.13 kg (SDPP3), 69.04 ± 2.24 kg (SDPP4) and 70.67 ± 2.11 kg (SDPP5). The mean height for each group was 1.56 ± 0.13 m, .62 ± 0.19 m and 1.63 ± 0.18 m, respectively. The mean body mass index (BMI) of the respondents was all in the overweight category (WHO, 1995). This can be seen in the results recorded where the mean BMI was 27.61 ± 0.61 kg/m2 (SDPP3), 26.24 ± 0.78 kg/m2 (SDPP4) and 26.47 ± 0.6 kg/m2 (SDPP5). In a study by Narayan and Abdul Rashid Khan (2007) [14] found out that the prevalence of overweight in two rural villagers in Northern Malaysia was 25.9%. There was also a study conducted among attendees of health clinics in Sepang district in Malaysia showed a high prevalence of overweight that was 31% [15]. The factors that could be causing the problem in this community could be due to inactive lifestyle and high fat intake.

3.2. Nutrient Intake and Energy Expenditure

Nutrient intakes were recorded for three days, where it constitutes of two days on weekdays and one day on weekend (Table 2). All subjects were called (by phone) by the researchers to ensure that the subjects drink and record their consumption of SDPP in the food record book as a proof of compliancy. The mean energy intake of each group while receiving SDPP supplement showed no significant different when compared to the control week. The amount of energy intake of the respondents was sufficient for them to perform their daily activities (1781± 84 - 1842 ± 116 kcal). Malaysian Adults Nutrition Survey (MANS) [16] conducted between the year 2002 and 2003 had reported a lower mean energy intake of Malay adults (1635 ± 14 kcal) and community that live in rural area (1653 ± 51 kcal) as compared to the mean energy intake from this study.

Table 2: Average of nutrient intake based on 3-days food record

	SDPP 2		SDPP 3		SDPP 4	
	Supplement	Control	Supplement	Control	Supplement	Control
Energy (Kcal/d)	1781 ± 84	1804 ± 56	1779 ± 92	1815 ± 81	1806 ± 96	1842 ± 116
Protein (g/d)	58 ± 4	62 ± 5	70 ± 1	71 ± 3	58 ± 5	55 ± 5
Carbohydrate (g/d)	279 ± 12	280 ± 6	270 ± 17	283 ± 13	278 ± 20	310 ± 15
Fat (g/d)	50 ± 2	53 ± 4	54 ± 7	52 ± 7	51 ± 3	54 ± 2
Cholesterol (mg/d)	225 ± 25	240 ± 19	230 ± 15	235 ± 21	218 ± 12	202 ± 17
Dietary Fiber (g/d)	21 ± 1	10 ± 1	25 ± 2	12 ± 1	26 ± 1	9 ± 1
Vitamin C (mg/d)	50 ± 5	24 ± 2	53 ± 2	14 ± 1	58 ± 2	20 ± 1
Energy expenditure (Kcal/d)	1752 ± 99	1777 ± 80	1808 ± 106	1822 ± 112	1840 ± 96	1835 ± 88

Values are expressed as mean ± standard error mean (SEM), n = 60.

The three-day physical activity records were analyzed for total energy expenditure throughout the supplementation weeks and control weeks for all respondents. Average energy expenditure of respondents was ranged between 1752 to 1840 kcal/day. There was no significant difference between energy intake and energy expenditure for all groups. This indicates that they have maintained their daily live activities. Throughout the 10 weeks study, none of them reported any changes in their food intake and loss of appetite.

3.3. Plasma lipid Profiles and Total Atioxidant Status

Fruits and vegetables containing a wide range of nutrients and phytochemicals have been associated with significant cardiovascular health benefits. SDPP has been shown to have dietary fibers, minerals, vitamin c and antioxidants that may lower the lipid profiles. The result in Table 3 presented the mean of lipid profiles (TC, TG, HDL and LDL) and total antioxidant status of the respondents throughout the study. In the present study, we found a significant decrease ($p \leq 0.05$) in TC after 4 weeks supplementation of SDPP. All three dosages of SDPP showed a decreasing trend of TC. The range of TC dropped in SDPP group was 20.75% - 30.1% after 4 weeks supplementation. The higher the dosages given to the subjects, the higher the TC level dropped. But, there was no significant difference (P > 0.05) found in TC when compared to all three dosages of SDPP. In this study, plasma triglycerides showed an inconsistency trend of changes. TG was only significantly lower ($p < 0.05$) compared with baseline values in group 4S SDPP (9.65%). As for HDL-C, dietary supplementation of 5 sachets SDPP for 4 weeks significantly increased HDL-C levels by 11.76% ($p < 0.05$) compared to baseline. A significant decreasing trend in LDL-C level was observed ($p < 0.05$) in this study. LDL-C decreased by 21.88% (3 sachets SDPP), 25.89% (4 sachets SDPP) and 27.41% (5 sachets SDPP) respectively after 4 weeks of supplementation compared to baseline. For TAS, the consumption of 3 sachets of SDPP/ day did not alter the plasma TAS concentration. But, for both 4 sachets and 5 sachets of SDPP supplementation groups showed an increased percentage in TAS compared to baseline although only group with 5 sachets performed a significant increased ($p < 0.05$) by 12.31% after 4 weeks of consumption. In contrast to control phase, the plasma TAS of group 4 and 5 sachets SDPP showed a significant reduction ($p < 0.05$) after a wash-out period. The result was in agreement with previous in-vivo study by Mohd Adzim Khalili et al, 2009 [17] and Halimah et al, 2009 [18]. The study by Mohd Adzim Khalili et al, 2009 was done using 30 Sprague Dawley rats (induced hypercholesterolemic) supplemented with freeze-dried red pitaya (0.5%, 0.83% and 1.17% per daily diet) for 5 weeks. From this study, they found that daily oral administration of red pitaya supplements showed a positive result on significantly reduced total cholesterol levels (49.14% - 59.06%) in plasma after 5 weeks of supplementation. There was also another similar study done by Halimah et al, 2009. The study used rats treated with 300 mg/kg (body weight) of peel and flesh aqueous extracts of *H. polyrhizus* fruit via force feeding for 10 days. The results showed that total cholesterol level was reduced significantly ($p<0.05$) in both hypercholesterolemic groups treated with peel and flesh extracts by 51.36% and 43.53% respectively.

Table 3: Plasma lipid profiles, glucose and total antioxidant status of respondents throughout the study

		BASELINE	SUPPLEMENT	WASH-OUT	CONTROL
TC	3S	5.06 ± 0.08	4.01 ± 0.11*	4.87 ± 0.83	5.02 ± 0.83#
	4S	5.12 ± 0.09	3.84 ± 0.16*	4.94 ± 0.12	5.03 ± 0.11
	5S	4.95 ± 0.09	3.46 ± 0.14*	4.78 ± 0.12	4.83 ± 0.11
TG	3S	1.27 ± 0.10	1.17 ± 0.09	1.29 ± 0.09	1.28 ± 0.09
	4S	1.14 ± 0.12	1.03 ±0.14*	1.08± 0.13	1.11 ± 0.13
	5S	0.98 ± 0.11	0.94 ± 0.09	1.19 ± 0.11	1.21 ± 0.12
HDL	3S	1.48 ± 0.04	1.54 ± 0.04	1.45 ± 0.04	1.41 ± 0.04
	4S	1.47 ± 0.05	1.55 ± 0.04	1.49 ± 0.05	1.44 ± 0.06
	5S	1.36 ± 0.05	1.52 ± 0.05*	1.38 ± 0.06	1.38 ± 0.05
LDL	3S	3.29 ± 0.10	2.57 ± 0.12*	3.12 ± 0.09	3.36 ± 0.11#
	4S	3.36 ± 0.11	2.49 ± 0.13*	3.25 ± 0.12	3.39 ± 0.14
	5S	3.32 ± 0.11	2.41 ± 0.14*	3.19 ± 0.12	3.17 ± 0.12
TAS	3S	1.33 ± 0.09	1.23 ± 0.06	1.08 ± 0.13	1.09 ± 0.14
	4S	1.08 ± 0.09	1.2 ± 0.07	1.23 ± 0.12	0.96 ± 0.06#
	5S	1.3 ± 0.07	1.46 ± 0.08*	1.25 ± 0.07	0.88 ± 0.08#

Values are expressed as mean ± SEM, n = 60. *Supplement were significantly different (p-value < 0.05) compared to baseline. #Supplement were significantly different (p-value < 0.05) compared to control.

Cholesterol-lowering effects of SDPP supplementation may be attributable to the high dietary fiber, vitamin C and minerals content, especially potassium, sodium, magnesium, zinc and iron. The reduction of total cholesterol level in groups with supplementation of SDPP may be due to the increased excretion of bile acid. The dietary components in red pitaya which have been reported to have a hypocholesterolemic effect through increased excretion of bile acids are soluble fiber, unsaturated fatty acids and minerals especially potassium, sodium, magnesium, phosphorus and zinc [19, 20]. In a study by Basu et al., 2009 [21] also reported the same trend where a total dietary fiber content of freeze-dried strawberry powder (8 g/day) reduced the cholesterol level.

4. Conclusion

Overall, the results of the present study suggest that SDPP, a concentrated source of dietary fiber, vitamins and minerals and antioxidant compounds is a novel dietary fruit supplement that has a bright future as one of the functional foods. In conclusion, regular consumption of spray dried *pitaya* powder (SDPP) did not result in significant weight gain. Our study shows the potential role of SDPP in lowering total and LDL-cholesterol ($p < 0.05$) in normocholesterolemic subjects. These observations therefore suggest that SDPP is a useful dietary component to prevent and/or ameliorate atherosclerosis. Further investigation is needed to establish the underlying mechanisms of this SDPP on hypocholesterolemic effects and better understanding of its role in contributing to the antioxidant systems. The use of this SDPP up to the dose of 100g/day showed no adverse effects on liver and kidney abnormalities during 4 weeks supplementation.

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