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THE EFFECTS OF GUAVA (*PSIDIUM GUAJAVA*) CONSUMPTION ON TOTAL ANTIOXIDANT AND LIPID PROFILE IN NORMAL MALE YOUTH

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ABSTRACT

Fruits contain a broad spectrum of nutrients, and many of which have antioxidant properties. Phenolic substances, vitamin A, vitamin C and minerals that are present in fruits displayed high antioxidant activity. Thes properties have been associated with the decreased risk of certain degenerative diseases. This study was conducted to determine the effects of guava (*Psidium guajava*) consumption on total antioxidant status and lipid profile (total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol) in normal male youth. This study was carried out over nine weeks, which was divided into three phases, that is, baseline (one week), treatment (four weeks) and control (four weeks). Blood samples were collected at the end of each phase for biochemical test. Total antioxidant status, glucose, lipid profile and antioxidant enzymes (glutathione peroxidase and glutathione reductase) were determined using Cobas Mira auto analyzer (Roche). Dietary intake in each phase was studied using 24-hours diet recall. There was a significant increase of total cholesterol, triglyceride and HDL-cholesterol during the treatment phase, compared to the baseline and control phases ($p < 0.05$). The increase of HDL-cholesterol was associated with the decreased risk of heart attack and cardiovascular disease. Although there was an increase in total cholesterol and triglyceride in the treatment phase compared to baseline and control phases, the increase was still in normal range. There was a significant increase of total antioxidants during the treatment phase, compared to the baseline and control phases ($p < 0.05$). There were trends of reduction for both glutathione peroxidase and glutathione reductase in the treatment phase as compared to baseline and control phases. However, the reduction was not statistically significant. The reduction of antioxidant enzymes was associated with decreased oxidative stress and decrease in free radical activities. The consumption of guava, therefore, could result in improved antioxidant status and lipid profile. Thus, it could reduce the risk of disease caused by free radical activities and high cholesterol in blood.

Keywords: guava, lipid profile, total antioxidant status

French

EFFETS DE LA CONSOMMATION DE LA GOYAVE (*PSIDIUM GUAJAVA*) SUR LE PROFIL TOTAL DES ANTIOXIDANTS ET DE LIPIDES CHEZ LES JEUNES DE SEXE MASCULIN

RÉSUMÉ

Les fruits contiennent une large gamme de substances nutritives, et la plupart d'entre eux sont caractérisés par des propriétés d'antioxydant. Les substances phénoliques, la vitamine A, la vitamine C et les minéraux que l'on trouve dans les fruits ont affiché une activité intense d'antioxydant. Ces propriétés ont été associées au risque réduit de certaines maladies dégénératives. Cette étude a été dirigée afin de déterminer les effets de la consommation de la goyave (*Psidium guajava*) sur la situation générale d'antioxydant et sur le profil des lipides (total du cholestérol, les triglycérides, le cholestérol-LDL et le cholestérol-HDL) des jeunes normaux de sexe masculin. Cette étude a été réalisée sur une période de plus de neuf semaines, qui a été divisée en trois phases, à savoir l'étude initiale (une semaine), le traitement (quatre semaines) et le contrôle (quatre semaines). Les échantillons de sang recueillis à la fin de chaque phase ont été soumis au test biochimique. La situation globale d'antioxydant, de glucose, du profil des lipides et des enzymes d'antioxydant (glutathione peroxidase et glutathione reductase) a été déterminée grâce à l'auto-analyseur Cobas Mira (Roche). La ration diététique de chaque phase a été étudiée en utilisant un régime de retrait de 24 heures. Une augmentation significative du total de cholestérol, triglycéride et cholestérol-HDL a été constatée pendant la phase de traitement, en comparaison aux phases initiale et de contrôle ($p < 0,05$). L'augmentation du cholestérol-HDL a été associée au risque réduit de crise cardiaque et de maladie cardio-vasculaire. Bien qu'il y avait une augmentation, dans l'ensemble, du cholestérol et du triglycéride dans la phase de traitement en comparaison avec les phases initiale et de contrôle, les accroissements se situaient encore dans les normes. Une augmentation significative du total des antioxydants pendant la phase de traitement a été constatée, en comparaison aux phases initiale et de contrôle ($p<0,05$). Des tendances de réduction pour glutathione peroxidase et glutathione reductase ont apparu dans la phase de traitement en comparaison aux phases initiale et de contrôle. Toutefois, la réduction n'était pas statistiquement considérable. La réduction des enzymes antioxydants a été associée aux diminutions des tensions oxydatives et à la réduction des activités radicales libres. Donc, la consommation de goyave pourrait résulter en une amélioration de la situation des antioxydants ainsi que celle du profil des lipides. De ce fait, il pourrait réduire le risque des maladies causées par les activités radicales libres et le niveau élevé de cholestérol dans le sang.

Mots clés: goyave, profil de lipide, situation totale d'antioxydant

INTRODUCTION

The human body naturally produces free radicals that cause oxidation in cells. This phenomenon exposes our body to many diseases such as cancer, cardiovascular disease and arthritis [1]. Besides that, free radicals or reactive oxygen species can cause chronic diseases such as trauma, skin and colon cancer. Injuries from free radical also causes atherosclerosis [2].

Now days, there are many dietary supplements associated with antioxidants such as, β-carotene, vitamin C, vitamin E and multivitamin. However, some communities are not aware that they can get cheaper and better antioxidants from natural sources. *Psidium guajava* or guava is very rich in antioxidants and vitamins. For every 100 gram of guava, there are 59.5 μg carotene, 9.9 μg retinol equivalent, 0.1 mg vitamin B_1, 0.05 mg vitamin B_2, 1.08 mg niacin, 6.76 g fibre and 151.4 mg vitamin C (ascorbic acid). Guava is also high in lutein, zeaxanthine and lycopene [3, 4].

Antioxidants physiologically act to prevent cellular component damage caused by free radicals. In the biological system, antioxidants are divided into four categories, namely; (a) endogenous (NADPH, NADH, glutathione, uric acid, bilirubin, metalloenzymes), (b) exogenous especially from diet (vitamin C, α-tocopherol, carotenoid), (c) metal-binding protein in the body such as albumin, transferrin, ferritin, and (d) antioxidant enzymes (superoxide dismutase, glutathione peroxidase, catalase) [5].

The effects of multi-nutrient supplementation on antioxidant defence systems in healthy human beings showed a significant increased in the plasma level of vitamin C, E and β-carotene, red blood cells, vitamin E as well as blood selenium. Besides that, the activity of catalase and glutathione peroxidase were increased but not for superoxide dismutase and blood glutathione [6].

Antioxidant profile in biological fluid and tissues can be used as markers for oxidative stress in humans. Antioxidants in plasma can be low compared to control in normal and abnormal condition; for examples due to diseases that were associated with free radical. Moreover, antioxidant in plasma can be used as a parameter for oxidative stress status [7]. A study by Youdim and Dean (2000), showed that dietary antioxidant can improve antioxidant enzymes and total antioxidant status that can prevent aging [8].

Fruits also have a high content of antioxidant minerals, vitamins and phytochemicals that can react as antioxidants. Consumption of fruits and vegetables has been associated with decreased risk of cancer and cardiovascular diseases. Short-term intervention of consuming high amounts of fruits and vegetables can increase plasma antioxidant concentration as well as reduce blood pressure [9]. Recent research reported that guava consumption in hypertensive and hypercholesterolemia are able to increase significantly the intake of carbohydrate, fibre, potassium and vitamin C. The increase of vitamin C intake was shown to be associated with increase of plasma ascorbic acid (45.3 %), high density lipoprotein-cholesterol (HDL-cholesterol) (8.9 %) and decrease of total cholesterol (11.9 %), triglyceride (8.1 %), systolic (5.0 %) and diastolic (4.6 %) [10].

This study was designed to study and evaluate the effects of consumption of guava on total antioxidant status and lipid profile in normal male youth.

MATERIALS AND METHODS

This study was conducted at the Institute Kemahiran Belia Negara, Dusun Tua, Hulu Langat, Selangor, Malaysia. The majority of the students at the institute are aged between 18 to 24 years old (mean 19 years old). 28 normal male youth, 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, but asked to incorporate guava (approximately 400 gram per day). (Table 1).

Subjects

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

i. Volunteer
ii. Aged between 18 to 24 years old
iii. Normal fasting blood glucose (3 – 7 mmol/l)
iv. Normal total cholesterol level (< 5.2 mmol/l)
v. Do not consume any medicine or supplements
vi. Free from disease

Study design

This was done as a pre and post experimental study. This study was conducted for nine weeks and divided into three phases: 1) baseline (one week), 2) treatment phase (four weeks) and 3) control phase (four weeks). Each subject would act as treatment and control.

Baseline phase (1 week)

Upon starting the study, the 28 subjects were asked to fill in the information questionnaire and 24-hour diet recall (two days) for diet study. Body weight, height and fasting blood sample were collected.

Treatment phase (4 weeks)

In this phase, approximately 400 g of 'Thai seedless' guava (Pertiwi Teguh Sdn. Bhd.) were added to each subjects' diet every day for 28 days. At the end of this phase, fasting blood samples were collected and filled the 24-hour diet recall (two days) for diet study.

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Control phase (4 weeks)

In this phase, respondents were required not to take guava for 28 days. At the end of this phase, fasting blood sample was collected and fill 24-hour diet recall (two days) for diet study.

Collection of blood samples

Venous blood samples (10 ml) were withdrawn from subjects following overnight fasting of at least 10 h. The blood samples were divided into three portions; 4 ml in plain tube, 3 ml in tube coated with EDTA, and 3 ml in tube with a mixture of potassium fluoride, sodium and EDTA. The mixture of potassium fluoride, sodium and EDTA was used to prevent glycolysis and prevent glucose deposition. The blood in the plain tube and potassium fluoride + sodium + EDTA tube were centrifuged at 3000 rpm for 15 minutes to get serum and plasma (Kubota 2100 centrifuge). Blood in EDTA tube was stored at—80 °C as whole blood, and so were all others.

Measurement of biochemical parameters

Plasma glucose, total serum cholesterol, serum triglyceride, serum HDL-cholesterol, serum antioxidant status (TAS), whole blood glutahione peroxidase and serum glutathione reductase were measured using Cobas Mira autoanalyzer. The kits were provided by Roche, U.K. Low density lipoprotein-cholesterol (LDL-cholesterol) was then estimared by calculation using the Friedwald formula [LDL-cholesterol = (total cholesterol –HDL-cholesterol) – (triglyceride / 2.2) [11].

Measurement body weight and height.

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

Diet study

Respondents were asked to fill a 24-hour diet recall form at the end for each phase. The data was converted into nutrient (calorie, carbohydrate, protein, fat, vitamins and minerals) using the DIET 4 programme.

Statistical analysis

Data was expressed as the mean ± S.D. The students *t*-test was used to analyse the significant difference between mean value, using Statistical Package for Social Sciences (SPSS) version 10.0. A *p* value of less than 0.05 was considered to be statistically significant.

RESULTS

28 respondents from the Institute volunteered to join this study (mean ± S. D.) (Table 1). Dietary intake plays a major role and is a main factor that influences the lipid profile and antioxidant levels in blood. Result from the diet study showed that there was a significant increase in the levels of protein, fat, carbohydrate, vitamin C, calcium and

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energy during the treatment phase compared to the baseline and control phases ($p < 0.05$) (Table 2).

There was a significant increase in glucose levels during this study ($p < 0.05$). Total cholesterol, triglyceride and HDL-cholesterol significantly increased during the treatment phase compared to baseline and control phases ($p < 0.05$). LDL-cholesterol also increased during treatment phase, but the increase was not significantly different compared to baseline and control phases (Table 3).

There was a significant increase in total antioxidant status during treatment phase compared to baseline and control phase ($p < 0.05$). Glutathione peroxidase and glutathione reductase activities did not differ during baseline, treatment and control phases (Table 4).

DISCUSSION

Epidemiological studies have shown that a high intake of fruits and vegetables is associated with reduced risk of cardiovascular diseases. Most fruits and vegetables contain significant quantities of polyphenolic compounds in addition to variable amounts of antioxidant vitamins including vitamin C, E and β-carotene. The antioxidant activity of whole fruits and vegetables is of great interest because oxidative processes have been shown to be vital in the initiation of atherosclerosis [12].

There was a significant increase in the blood glucose levels during the treatment and control phases compared to the baseline ($p < 0.05$). The lower level of glucose during treatment phase compared to control phase was probably associated with high fibre content in guavas. Every 100 g of guava contains 6.76 gram fibre [3]. In 'Thai Seedless' guava, there is 4.9 % of dietary fibre from edible proportion [13]. On the dry weight basis, Thai seedless guavas contain 37.2 % total dietary fibre, 10.1 % dissolved dietary fibre, 9.2 % cellulose and 5 % lignin [12]. Fruits that have high fibre content are able to control glycaemia by reduce the absorption of glucose in the intestine [14].

Biochemical tests on lipid profile showed a significant increase in total cholesterol, triglyceride and HDL-cholesterol in treatment phase, compared to baseline and control phases ($p < 0.05$). But, there was no significant difference of LDL-cholesterol in treatment phase compared to baseline phase but a significant increase compared to control phase ($p < 0.05$). The increase in of HDL-cholesterol was associated with decreased risk of heart attack and cardiovascular disease. Although there was an increase in total cholesterol and triglyceride in the treatment phase compared to baseline and control phases, the increased amounts were still within the normal range. Increase in total cholesterol was associated with high intake of fat and calorie during treatment phase compared to baseline and control phases ($p < 0.05$).

There was a significant increase in total antioxidant status during treatment phase compared to baseline and control phases ($p < 0.05$). The increase in total antioxidant status was probably due to increased antioxidant consumption contributed from guava which has been reported to be high in antioxidant vitamins such as ascorbic acid, β-carotene, lutein, zeaxanthin and lycopene [3, 4]. The increase in of total antioxidant status has been associated with decreased risk of diseases mediated by free radicals such as cancer. Antioxidant in plasma can act as a good indicator of oxidative stress in humans [7].

There were some reductions of glutathione peroxidase and glutathione reductase but not significant in treatment phase compared to baseline and control phases. The reduction of antioxidant enzymes were associated with decreased oxidative stress and free radical activities. This condition may be due to adaptive mechanism by antioxidant enzymes that can be induced by mild oxidative stress. The lower the oxidative stress, the lower antioxidant enzymes were produced in the body.

CONCLUSION

In this study, guava consumption resulted in significant increase in the levels of total antioxidants and reduced oxidative stress, as indicated by decreased levels of antioxidant enzymes (glutathione peroxidase and glutathione reductase). Besides that, consumption of guava could increase the level of HDL-cholesterol (lipoprotein that carried cholesterol from tissues to lower) significantly. Finally conclusion, consumption of guava is able to reduce oxidative stress and improve lipid profile. Thus, likely to reduce the risk of diseases caused by free radical activities and high blood cholesterol.

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Table 1. Characteristics of human subjects used to investigate the effect of consuming Thai seedless guava in normal male youth (mean ± S. D.)

Details (n=28)	**Mean ± S. D.**
Age (years)	19.25 ± 1.90
Weight (kg)	56.22 ± 9.57
Height (cm)	165.73 ± 4.32
BMI (kg/m^2)	20.47 ± 3.37

Table 2. Comparison of dietary intake during baseline, treatment and control phase

	Baseline	**Treatment**	**Control**
Energy (kcal)	2192.23 ± 311.54	2547.91 ± 178.68*	2180.14 ± 199.95
Protein (g)	97.13 ± 19.56	103.43 ± 24.37*	95.00 ± 10.50
Fat (g)	53.90 ± 9.31	68.02 ± 9.78*	54.68 ± 8.22
Carbohydrate (g)	334.13 ± 55.85	371.80 ± 43.53*	317.00 ± 42.43
Vitamin A (µg retinol equivalent)	674.63 ± 226.94	944.70 ± 252.99	850.14 ± 228.47
Vitamin C (mg)	28.83 ± 10.14	761.89 ± 34.14*	32.50 ± 10.08
Iron (mg)	11.30 ± 3.19	17.77 ± 5.07	15.07 ± 6.75
Calsium (mg)	255.55 ± 67.13	513.18 ± 82.10*	329.39 ± 110.56

* Significantly different ($p<0.05$) compared to baseline and control.

Table 3. Comparison of glucose level and lipid profile (total cholesterol, triglycerise, LDL-cholesterol and HDL-cholesterol) during baseline, treatment and control phase (mmol/l)

	Baseline (First week)	**Treatment (Fifth week)**	**Control (Ninth week)**
Glucose	4.43 ± 0.62	5.36 ± 1.19*	6.71 ± 1.25
Total cholesterol	3.78 ± 0.63	4.60 ± 0.97*	3.60 ± 0.51
Triglyceride	0.48 ± 0.27	1.34 ± 0.37*	0.89 ± 0.47
LDL-cholesterol	2.63 ± 0.59	2.75 ± 0.77	2.32 ± 0.53
HDL-cholesterol	0.92 ± 0.16	1.36 ± 0.37*	0.88 ± 0.22

* Significantly different ($p < 0.05$) compared to baseline and control.

Table 4. Comparison of total antioxidant status, glutathione peroxidase and glutathione reductase activities during baseline, treatment and control phase

	Baseline (First week)	**Treatment (Fifth week)**	**Control (Ninth week)**
Total antioxidant status (mmol/l)	1.24 ± 0.14	1.78 ± 0.22*	1.35 ± 0.21
Glutathione peroxidase (U/I)	458.21 ± 156.3	451.10 ± 179.56	466.32 ± 145.61
Glutathione reductase (U/I)	82.22 ± 48.96	80.15 ± 35.94	85.06 ± 26.00

- Significantly different ($p<0.05$) compared to baseline and control.

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