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Effects of Pink Guava (*Psidium guajava*) Puree Supplementation on Antioxidant Enzyme Activities and Organ Function of Spontaneous Hypertensive Rat

(Kesan Suplementasi Puri Jambu Batu Merah (*Psidium guajava*) ke Atas Aktiviti Enzim Antioksidan dan Fungsi Organ Tikus Hipertensi Spontan)

NORAZMIR MD NOR* & AYUB MOHD YATIM

ABSTRACT

This study was aimed to determine the effects of pink guava (Psidium guajava) *puree supplementation on enzyme activities, kidney and liver function tests of Spontaneous Hypertensive Rats (SHR). Twenty-four male SHR were divided into four groups (control, CG (distilled water); low dosage group, LDG (0.5 g/kg body weight); medium dosage group, MDG (1.0 g/kg body weight); high dosage group, HDG (2.0g/kg body weight)). The rats were given pink guava puree via force-feeding and fed rat pellets ad libitum for 28 days in individual cages at 25±2°C. At the end of experiment, the rats were fast overnight (12 to14 h) and euthanized under an anesthetic condition with ether, and blood was collected from the portal vein or posterior vena cava. The specific activities of glutathione peroxidase (GPx) was significantly higher in LDG (2332.5±81.8 U/L), MDG (2424.8±97.1 U/L) and HDG (2594.6±82.8 U/L) respectively, as compared to CG (2171.8±65.9 U/L). Significant differences were also seen in glutathione reductase (GR) activities among all treated groups (LDG (132.5±11.8 U/L), MDG (141.5±16.4 U/L), HDG (148.8±13.2 U/L) compared to CG (126.1±14.2 U/L)). Liver function tests for total antioxidant status (TAS), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and γ-glutamyl transpeptidase (GGT) showed significant differences in the treated group compared to control group. In conclusion, this study shows pink guava puree supplementation increase antioxidant enzyme activity in SHR's blood concentration.*

Keywords: Antioxidant; enzyme activity; organ functions; pink guava; spontaneous hypertensive rats

ABSTRAK

Kajian dijalankan untuk menentukan kesan pemberian suplemen puri jambu batu merah (Psidium guajava) *ke atas aktiviti enzim, dan ujian kefungsian ginjal dan hati tikus teraruh hipertensi (SHR). Sebanyak 24 ekor SHR dibahagi kepada 4 kumpulan (kawalan, CG (air suling); dos rendah, LDG (0.5 g/kg berat badan); dos sederhana, MDG (1.0 g/kg berat badan); dos tinggi, HDG (2.0 g/kg berat badan)). Tikus diberi puri jambu batu merah melalui suap-paksa dan pelet tikus secara ad libitum selama 28 hari dan diletakkan dalam sangkar individu pada suhu 25±2°C. Akhir kajian, tikus dipuasa semalaman (12-14 jam) dan dibiuskan di bawah pengaruh eter, dan darah diambil dari vena kava posterior. Aktiviti spesifik bagi glutation peroksida (GPx) adalah tinggi secara signifikan dalam LDG (2332.5±81.8 U/L), MDG (2424.8±97.1 U/L) dan HDG (2594.6±82.8 U/L) masing-masing, berbanding CG (2171.8±65.9 U/L). Terdapat perbezaan signifikan pada aktiviti glutation reduktase (GR) dalam kumpulan rawatan (LDG (132.5±11.8 U/L), MDG (141.5±16.4 U/L), HDG (148.8±13.2 U/L) berbanding CG (126.1±14.2 U/L)). Ujian fungsi hati bagi status antioksidan total (TAS), alanin aminotransferase (ALT), aspartat aminotransferase (AST), laktat dehidrogenase (LDH) dan γ-glutamil transpeptidase (GGT) menunjukkan perbezaan signifikan dalam kumpulan rawatan berbanding kumpulan kawalan. Kajian menunjukkan suplementasi puri jambu batu merah meningkatkan aktiviti enzim antioksidan dalam darah tikus hipertensi spontan.*

Kata kunci: Antioksidan; aktiviti enzim; fungsi organ; jambu batu merah; tikus hipertensi spontan

INTRODUCTION

Guava (*Psidium guajava*) is widely cultivated and its fruit is popular and well-known. Red-fleshed Brazilian guava has several carotenoids such as phytofluene, ß-carotene, ß-cryptoxanthin, lycopene, rubixanthin and lutein (Thaipong et al. 2006). Guava was also used as a hypoglycemic agent in folk medicine. The leaves and skin of the fruit have greater effects. Cheng and Yang (1983) proved that guava juice exhibited hypoglycemic effects in mice. Interestingly, the decreased serum glucose level of infusions from the African mistletoe (*Loranthus bengwensis* L.) parasite on guava trees was more affected than that prepared from mistletoe parasitic on other trees (Obatomi et al. 1994). In other studies, the anti-diarrheal (Lutterodt 1989) bio-antimutagenic (Matsuo et al. 1994), antipyretic (Olajide et al. 1999), anti-microbial (Jaiarj et al. 1999) and anti-hypertensive (Ayub et al. 2010) properties of guava have been demonstrated. However, information concerning the enzyme activity of pink guava puree related to hypertension is unavailable. The objectives of this

study were to determine the effects of pink guava puree supplementation on enzyme activities, and kidney and liver functions of Spontaneous Hypertensive Rats (SHR).

Materials and Methods

PINK GUAVA PUREE SAMPLE

Pink guava (*Psidium guajava*) puree from variety *Beaumont Semenyih* was obtained directly from Golden Hope Food & Beverages Sdn. Bhd. Table 1 shows the content of the pink guava puree used in the experiment. The puree which was packed in an aseptic bag was stored immediately at -70ºC until the study was carried out. Once opened, the puree was repackaged into glass bottles of about 100 ml each and stored again at -70ºC until used.

TABLE 1. Nutrient content of pink guava (*Psidium guajava*) puree

Proximate analysis		Value
Macronutrients	Energy	147 kJ
	Carbohydrate	7.00%
	Protein	1.70%
	Fat	0.0%
	Ash	0.50%
Micronutrients	Vitamin A	108.50 mg/100 g
	Vitamin B1	BDL
	Vitamin B2	BDL
	Vitamin C	73.10 mg/100 g
	Niacin	13.30 mg/100 g
	Phosphorus	0.77 mg/100 g
	Calcium	131.66 ppm
	Iron	20.44 ppm
	Potassium	149.80 ppm
	Sodium	139.95 ppm

BDL: Below detection limit
(Source: Ayub et al. 2010)

EXPERIMENTAL ANIMALS

A total of 24 male SHR rats each weighing between 200-250 g obtained from University of Malaya's Animal House were used for the study. SHR rats were fed with a standard rat chow diet and water *ad libitum* for 28 days in individual cages. All SHR rats were acclimatized to the animal facility for one week in an air-conditioned room 25±2°C to a 12:12 hours light (7:30 am to 7:30 pm hour)/ dark cycle before starting the experiment. In the study, SHR rats were divided into four groups of six rats per group. Control group (CG) was given distilled water by an oral feeding. Low dosage group (LDG), medium dosage group (MDG) and high dosage group (HDG) were given a puree of pink guava (*Psidium guajava*) orally at doses of 0.5, 1.0 and 2.0 g/kg body weight, respectively.

ANALYTICAL PROCEDURES

After 28 days of oral feedings, SHR rats were fasted overnight (12 to 14) and euthanized under an anesthetic condition using ethyl ether. Blood was collected from the portal vein or posterior vena cava and transferred into tube containing anticoagulant solution, EDTA to get the plasma fraction. The whole blood was used to measured enzyme activities such as glutathione peroxidase and glutathione reductase. Serum was obtained by collecting blood in non-EDTA tube. The serum was used for determine kidney function test and liver function test. Plasma and serum samples were kept at -20ºC. The reagents were supplied by Randox. All analysis was done using Blood Chemical Analyzer (*Vitalab Selectra E, UK*) in Food Technology Centre laboratory, MARDI.

STATISTICAL ANALYSIS

Results are expressed as mean values ± standard deviation. Comparison of means using a significance level of $p<0.05$, was performed by one-way analysis of variance (ANOVA) using the SAS System for Windows, version 6.12 software.

Results and Discussion

EFFECT OF PINK GUAVA PUREE ON ENZYME ACTIVITIES

Oral administration of pink guava (*Psidium guajava*) puree did not induce mortality up to the highest dose, which was 2.0 g/kg body weight. No treated SHR rats showed toxic signs such as nose bleeding, fur loss, diarrhea and death throughout the observation period. The administration of the highest dose used in the experiment does not show any toxicity effects can be considered as safe (WHO 1992). Hadijah et al. (2004) also reported similar results in acute and sub-chronic study of *Morinda citrifolia* extract. Table 2 shows the specific activities of glutathione peroxidase (GPx) was significantly higher in LDG (2332.5±81.8 U/L), MDG (2424.8±97.1 U/L) and HDG (2594.6±82.8 U/L), respectively, as compared to CG (2171.8±65.9 U/L). Significant differences were also seen in glutathione reductase (GR) activities among all treated groups (LDG (132.5±11.8 U/L), MDG (141.5±16.4 U/L), HDG (148.8±13.2 U/L) compared to CG (126.1±14.2 U/L)). Prince and Menon (1999) showed that oral administration of aqueous *Tinospora cordifolia* root extract, an indigenous plant used as medicine in India, resulted in a decreased level of TBARS and an increase in the levels of glutathione, which is similar to this study.

EFFECT OF PINK GUAVA PUREE ON KIDNEY FUNCTION

Kidney is the second organ most frequently affected by any compound (Marshall 2000). Therefore, renal functions can be assessed by measuring the concentration of creatinine and urea in plasma (Moshi et al. 2001). Previous report showed that some herbal preparations used in long period

TABLE 2. Effect of pink guava puree supplementation on enzyme activities of Spontaneous Hypertensive Rats

	CG (distilled water)	LDG (0.5 g/kg bw)	MDG (1.0 g/kg bw)	HDG (2.0 g/kg bw)
Glutathione peroxidase (U/L)	2171.8±65.9[a]	2332.5±81.8[b]	2424.8±97.1[c]	2594.6±82.8[d]
Glutathione reductase (U/L)	126.1±14.2[a]	132.5±11.8[b]	141.5±16.4[c]	148.8±13.2[d]

Superscripts with different letters are significantly different at p<0.05 within the same row; n=6

are associated with kidney injury (Kadiri et al. 1999). There were no significant changes in urea concentrations in all groups as shown in Table 3. This indicates that pink guava puree did not affect the normal concentrations of urea and creatinine. Plasma urea and creatinine concentrations are often used as an index of renal glomerular function and will be increased in renal injuries (Marshall 2000). Urea is synthesized in the liver, primarily as by-product of the deamination of amino acids. Creatinine is a by-product from muscle mass will affect its concentration in blood (Vaughn 1999).

EFFECT OF PINK GUAVA PUREE ON LIVER FUNCTION

The activities of serum enzyme (AST, ALT, LDH and GGT), total protein and albumin concentrations are summarized in Table 4. These parameters are commonly used to evaluate the status of liver function (Lamela et al. 1986). Liver function test is crucial because liver is the central organ in detoxification of compounds (Heywood 1983). There are a number of circumstances that the measurement of enzyme activities in body fluids such as blood, may be of diagnostic values. In general, enzymes provide an excellent marker of tissue damage. Organ or tissue damage causes the release of increased amounts of many enzymes into the blood stream (Marshall 2000). Vaughn (1999) reported that the activities of most enzymes normally detectable in blood remain constant in healthy and normal person. The result of total protein and albumin concentrations were not affected by the pink guava puree supplementation. This shows that the synthesis of protein in the SHR rat's liver is not influenced by the supplementation. Similar results were also obtained in the toxicity studies of *Centella asiatica* (Lucia et al. 1997). A healthy liver is so crucial for protein metabolism since liver disease is frequently associated with alterations in proteins and disturbances of protein metabolism (Marshall 2000). Total protein and albumin concentrations will be decreased by inadequate synthesis due to liver disease (Datta et al. 1999). Liver function tests for total antioxidant status, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase and γ-glutamyl transpeptidase showed significant differences in the treated group compared to control.

TABLE 3. Effect of pink guava puree supplementation on kidney function test in Spontaneous Hypertensive Rats

	CG (distilled water)	LDG (0.5 g/kg bw)	MDG (1.0 g/kg bw)	HDG (2.0 g/kg bw)
Urea (mmol/L)	58.06 ± 6.80[a]	57.79 ± 0.68[a]	52.84 ± 1.56[a]	48.34 ± 2.44[a]
Creatinine (mg/dl)	8.05 ± 0.81[a]	8.76 ± 2.28[a]	8.43 ± 2.17[a]	8.09 ± 2.06[a]

Superscripts with different letters are significantly different at p<0.05 within the same row; n=6

TABLE 4. Effect of pink guava puree supplementation on liver function test in Spontaneous Hypertensive Rats

	CG (distilled water)	LDG (0.5 g/kg bw)	MDG (1.0 g/kg bw)	HDG (2.0 g/kg bw)
TAS (mmol/L)	1.60 ± 0.13[a]	1.49 ± 0.25[ab]	1.41 ± 0.45[a]	1.33 ± 0.65[b]
ALT (U/L)	46.75 ± 10.86[a]	56.75 ± 16.43[b]	54.13 ± 10.62[a]	51.50 ± 4.81[a]
AST (U/L)	123.50 ± 20.09[a]	153.75 ± 40.00[b]	136.75 ± 25.95[a]	119.75 ± 11.89[a]
LDH (U/L)	1528.50±274.01[a]	1229.50±556.41[a]	979.63±411.28[a]	729.75±266.14[b]
TPro (g/L)	78.17 ± 5.36[a]	80.60 ± 7.78[a]	78.68 ± 5.21[a]	76.75 ± 2.63[a]
Albumin (g/L)	35.80 ± 1.80[a]	35.50 ± 2.51[a]	34.24 ± 3.08[a]	32.98 ± 3.65[a]
Globulin (g/L)	42.50 ± 4.04[a]	44.75 ± 7.61[a]	44.38 ± 6.11[a]	44.00 ± 4.60[a]
A/G ratio	0.85 ± 0.07[a]	0.80 ± 0.13[a]	0.78 ± 0.14[a]	0.77 ± 0.15[a]
GGT (U/L)	2.70 ± 0.84[a]	0.79 ± 0.90[b]	1.91 ± 1.10[a]	3.03 ± 1.29[a]

Superscripts with different letters are significantly different at p<0.05 within the same row; n=6
TAS: Total antioxidant status
ALT: Alanine transaminase
AST: Aspartate aminotransferase
LDH: Lactate dehydrogenase
GGT: γ-glutamyl transpeptidase
TPro: Total protein

CONCLUSIONS

Pink guava (*Psidium guajava*) puree showed no toxic outcome in this study. The supplementation doses of pink guava puree ranging from 0.5–2.0 g/kg of body weight for 4 weeks did not produce any abnormalities in blood biochemical parameters. The specific activities of GPx and GR were significantly higher in LDG, MDG and HDG, respectively, as compared to CG. In conclusion, this study showed that pink guava puree supplementation increase enzyme activity in SHR's blood concentration.

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Norazmir Md Nor*
Department of Nutrition & Dietetics
Faculty of Health Sciences
Universiti Teknologi MARA
Puncak Alam Campus 42300 Puncak Alam
Selangor D.E.
Malaysia

Ayub Mohd Yatim
Deceased

*Corresponding author; email: azmir2790@puncakalam.uitm.edu.my

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