# EFFECTS OF PINK GUAVA (*PSIDIUM GUAJAVA*) PUREE SUPPLEMENTATION ON ANTIOXIDANT ENZYMES ACTIVITIES AND ORGANS FUNCTIONS OF SPONTANEOUS HYPERTENSIVE RAT

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ABSTRACT

This study was aimed to determine the effects of pink guava puree supplementation on enzyme activities, and 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 fasted overnight (12 to14-hours) 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)]. Kidney function test showed significant differences in HDG's urea concentration (48.34 ± 2.44 mmol/l) as compared to CG (58.06±6.80 mmol/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 (*Psidium guajava*) puree supplementation increase enzyme activity in SHR's blood concentration.

***Keywords**: pink guava, antioxidant, enzyme activity, organ functions, spontaneous hypertensive rats*

## 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, Bikomo, & Temple, 1994). In other studies, the anti-diarrheal (Lutterodt, 1989) bio-antimutagenic (Matsuo et al., 1994), antipyretic (Olajide, Awe, & Makinde, 1999) and anti-microbial (Jaiarj et al., 1999) 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 effect of pink guava puree supplementation on enzyme activities, and kidney and liver functions of Spontaneous Hypertensive Rats (SHR).

## MATERIAL AND METHODS

### *Pink guava puree sample*

Pink guava (*Psidium guajava*) puree from variety *Beaumont Semenyih* obtained directly from Golden Hope Food & Beverages Sdn. Bhd. 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.

### *Experimental animals*

A total of 24 male *Spontaneous Hypertensive Rats (SHR)* each weighing between 200-250 g obtained from Animal House (University of Malaya) 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-hour 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 to14-hours) 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 glutahione 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*

The significant differences between the control and puree-treated groups were analyzed using SPSS software version 16. All mean values are expressed as group means ± standard error of mean. The minimal level of significance accepted was $p<0.05$.

## 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. All treated SHR rats did not show any 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). Similar results were also reported by Hadijah et al. (2004) in acute and subchronic study of *Morinda citrifolia* extract. Table 1 showed the specific activities of glutahione 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.

**Table 1**. 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

*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 are associated with kidney injury (Kadiri et al. 1999). There were no significant changes in urea concentrations in all groups as shown in Table 2. 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).

**Table 2**. 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

*Effect of pink guava puree on liver function*

The activities of serum enzyme (AST, ALT, ALP, LDH and GGT), total protein and albumin concentrations are summarized in Table 3. 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 fairly constant in healthy and normal person. The result of total protein and albumin concentrations were also 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 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) | $1.60 \pm 0.13^{a}$ | $1.49 \pm 0.25^{ab}$ | $1.41 \pm 0.45^{a}$ | $1.33 \pm 0.65^{b}$ |
| ALT (U/l) | $46.75 \pm 10.86^{a}$ | $56.75 \pm 16.43^{b}$ | $54.13 \pm 10.62^{a}$ | $51.50 \pm 4.81^{a}$ |
| AST (U/l) | $123.50 \pm 20.09^{a}$ | $153.75 \pm 40.00^{b}$ | $136.75 \pm 25.95^{a}$ | $119.75 \pm 11.89^{a}$ |
| LDH (U/l) | $1528.50 \pm 274.01^{a}$ | $1229.50 \pm 556.41^{a}$ | $979.63 \pm 411.28^{a}$ | $729.75 \pm 266.14^{b}$ |
| Total Protein (g/l) | $78.17 \pm 5.36^{a}$ | $80.60 \pm 7.78^{a}$ | $78.68 \pm 5.21^{a}$ | $76.75 \pm 2.63^{a}$ |
| Albumin (g/l) | $35.80 \pm 1.80^{a}$ | $35.50 \pm 2.51^{a}$ | $34.24 \pm 3.08^{a}$ | $32.98 \pm 3.65^{a}$ |
| Globulin (g/l) | $42.50 \pm 4.04^{a}$ | $44.75 \pm 7.61^{a}$ | $44.38 \pm 6.11^{a}$ | $44.00 \pm 4.60^{a}$ |
| A/G ratio | $0.85 \pm 0.07^{a}$ | $0.80 \pm 0.13^{a}$ | $0.78 \pm 0.14^{a}$ | $0.77 \pm 0.15^{a}$ |
| GGT (U/l) | $2.70 \pm 0.84^{a}$ | $0.79 \pm 0.90^{b}$ | $1.91 \pm 1.10^{a}$ | $3.03 \pm 1.29^{a}$ |

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

## CONCLUSION

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. Liver function tests for TAS, ALT, aspartate

AST, LDH and GGT showed significant differences in the treated group compared to control group. In conclusion, this study shows pink guava puree supplementation is considered safe and can increase enzyme activity in SHR's blood concentration.

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