Sains Malaysiana 40(2)(2011): 147–154

# Suplemen Puri Jambu Batu Merah (*Psidium guajava*) ke Atas Tikus Teraruh Obesiti: Kesan Terhadap Aktiviti Enzim Antioksidan, Fungsi Ginjal dan Fungsi Hati

(Pink Guava (*Psidium guajava*) Puree Supplement on High Fat Diet-Induced Obese Rats: Effects on Antioxidant Enzyme Activities, Kidney and Liver Functions)

AYUB MOHD YATIM & NORAZMIR MD NOR*

ABSTRAK

*Kajian ini dijalankan untuk menentukan kesan suplemen puri jambu batu merah (*Psidium guajava*) ke atas aktiviti enzim antioksidan, fungsi ginjal dan fungsi hati pada tikus teraruh obesiti. Sebanyak 30 ekor tikus Sprague-Dawley jantan dibahagi 5 kumpulan (kawalan negatif dan positif, CN dan CP (air suling); dos rendah, LDG (500 mg/kg bb); dos sederhana, MDG (1000 mg/kg bb); dos tinggi, HDG (2000 mg/kg bb). Suplemen puri jambu merah diberi bersama diet tinggi lemak selama 6 minggu. Pada akhir kajian, tikus dipuasa semalaman dan dibedah untuk kajian biokimia darah. Pengurangan signifikan dalam berat badan diperhatikan dalam kumpulan rawatan berbanding CN dan CP. Aktiviti spesifik seperti glutation peroksidase (GPx), glutatione redutase (GR) dan superoksida dismutase (SOD) pada tikus teraruh obesiti meningkat berbanding CN. Ujian fungsi ginjal bagi kepekatan urea menurun secara signifikan pada LDG (4.28±0.69 mmol/L), MDG (4.35±0.87 mmol/L) dan HDG (3.85±0.71 mmol/L) berbanding CN (7.02±1.81 mmol/L) masing-masing. Ujian fungsi hati bagi protein total, globulin, nisbah AG dan alanin aminotransferase (ALT) menunjukkan perbezaan signifikan LDG, MDG dan HDG berbanding CN. Nilai jumlah protein bagi MDG (72.67±3.65 g/L) dan HDG (76.00±2.49 g/L) lebih rendah berbanding CN (80.11±1.98 g/L). Nilai globulin bagi LDG (34.17±3.43 g/L), MDG (32.17±1.83 g/L) dan HDG (35.00±3.41 g/L) juga lebih rendah berbanding CN (39.67±0.82 g/L). Nilai nisbah AG bagi LDG (1.22±0.16), MDG (1.28±0.07) dan HDG (1.19±0.14) berbeza secara signifikan dengan CN (1.03±0.08). Nilai ALT bagi LDG (55.83±15.12 U/L), MDG (50.67±22.65 U/L) dan HDG (57.50±8.48 U/L) lebih rendah berbanding CN (77.00±16.26 U/L), masing-masing. Kesimpulannya, suplemen puri jambu merah bermanfaat dalam meningkatkan aktiviti enzim antioksidan dan mencegah komplikasi obesiti yang berhubungkait dengan fungsi ginjal dan hati.*

*Kata kunci: Aktiviti enzim; fungsi ginjal; fungsi hati; jambu batu; profil urin*

ABSTRACT

*In this study, the effect of pink guava (*Psidium guajava*) puree supplementation on antioxidant enzyme activities, kidney and liver functions in High Fat Diet (HFD)-induced obese rats was investigated. Thirty male Sprague–Dawley rats were divided into control negative (CN) fed with rat pellet; control positive, low, medium and high dose group (CP, LDG, MDG and HDG) were fed High Fat Diet-AIN93G, respectively. CN and CP were given distilled water; while the treated group were given the aqueous puree, at concentration of 500, 1000 and 2000 mg/kg body weight, dissolved in distilled water were administered orally via a drinking bottle, respectively. Pink guava puree was supplemented with the HFD diet for six weeks. The rats were fasted overnight and euthanized under an anesthetic condition with ethyl ether, and blood was collected from the posterior vena cava at the end of experiment. A significant reduction in body weight was observed in the treated groups as compared to CN and CP group. Specific activities of glutathione peroxidase (GPx), glutathione reductase (GR) and superoxide dismutase (SOD) of the HFD-induced obese rats were significantly increased in comparison with the CN group. Kidney function test of urea concentration were significantly decreased in HFD-induced obese rats; LDG (4.28±0.69 mmol/L), MDG (4.35±0.87 mmol/L) and HDG (3.85±0.71 mmol/L) as compared to CN (7.02±1.81 mmol/L), respectively. Liver function tests for total protein, globulin, A:G ratio and alanine aminotransferase (ALT) showed significant differences in the treated group compared to CN group. Total protein of MDG (72.67±3.65 g/L) and HDG (76.00±2.49 g/L) were significantly lower compared to CN (80.11±1.98 g/L). Globulin value for LDG (34.17±3.43 g/L), MDG (32.17±1.83 g/L) and HDG (35.00±3.41 g/L) were significantly lower compared to CN (39.67±0.82 g/L). A:G ratio LDG (1.22±0.16), MDG (1.28±0.07) and HDG (1.19±0.14) significantly different compared to CN (1.03±0.08). ALT value for LDG (55.83±15.12 U/L), MDG (50.67±22.65 U/L) and HDG (57.50±8.48 U/L) were significantly lower compared to CN (77.00±16.26 U/L), respectively. In conclusion, pink guava puree supplements seem to be beneficial for increased antioxidant enzyme activities and correcting/ preventing obesity complications in kidney and liver functions.*

*Keywords: Enzyme activities; kidney; liver; pink guava; urine profile*

## INTRODUCTION

Obesity have reached epidemic proportions throughout the world (James 2004) and it is associated with increased mortality, increased cardiovascular diseases, diabetes, colon cancers and gall bladder disease (Olusi 2002). Epidemiological studies of cancer and cardiovascular disease suggest that consumption of fruits, vegetables and plant-derived beverages is correlated with reduced risk of chronic disease (Appel et al. 1997). The benefits of plant-based foods may be a consequence of bioactive phytochemicals found in these foods. Phytochemicals include a wide variety of non-nutritive plant constituents that have diverse biochemical activities, including antioxidant properties (Norazmir et al. 2010).

Obesity also generates reactive oxygen species, which in turn cause lipid peroxidation and membrane damage. Lipid peroxidation is a free radical-generating process, which occurs on every membranous structure of the cell. Free radicals are known to be involved in a number of human pathologies including atherosclerosis (Steinberg 1997), cancer (Cerutti 1994) and hypertension (Russo et al. 1998). The increase of oxygen free radicals in obesity could be due to an increase of blood glucose level, since an autoxidation generates free radicals (Davi et al. 2005). Previous studies have reported that lipid peroxidation in liver, kidney and brain of rats was increased (Latha et al. 2003). Concerning to the changes in lipid peroxidation, the obese tissue showed decreased activity of the key antioxidants glutathione peroxidase (GPx), glutathione reductase (GR) and superoxide dismutase (SOD), which play an important role in scavenging the toxic intermediate of incomplete oxidation. The decrease in the activity of these antioxidants can lead to an excess availability of the superoxide anion and hydrogen peroxide in biological systems, which in turn generate hydroxyl radicals resulting in initiation and propagation of lipid peroxidation.

Oxidative stress is thought to contribute to the development of a wide range of diseases including Alzheimer's disease (Nunomura et al. 2006), Parkinson's disease (Wood et al. 2006), the pathologies caused by diabetes (Davi et al. 2005), rheumatoid arthritis (Hitchon & El-Gabalawy 2004) and neurodegeneration in motor neuron diseases (Cookson & Shaw 1999). Although oxidative stress generally seems to contribute to chronic diseases via a lifetime accumulation of oxidative events, systems for studying the role of dietary antioxidants *in vivo* generally include imposition of oxidative stress so that responses can be studied in a reasonably short time frame. Decades of research on oxidative stress have contributed to our understanding of mechanisms that underlie the health benefits and the potential dangers of cardiovascular disease (Wiseman & Halliwell 1996).

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). Previous study by Asmah et al. (2006) showed that 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. In previous study, it confirmed that pink guava have showed anti-hypertensive properties (Ayub et al. 2010).

Although animal studies in rats have shown that obesity is associated with increased myocardial oxidative stress (Vincent et al. 1999) and increased lipid peroxidation (Dobrian et al. 2000), to the best of our knowledge there is no report in the literature of the effect of pink guava puree supplement on antioxidant enzyme activities, kidney function test and liver function test. Therefore, pink guava puree thus represents a useful way to study the potential of its effect, including acting on antioxidant enzyme activities that may affect kidney and liver functions of High Fat Diet-induced obese rats.

## MATERIALS AND METHODS

### PINK GUAVA PUREE SUPPLEMENTATION

Pink guava (*Psidium guajava*) puree from combination of variety *Beaumont Semenyih* and *Beaumont Sungkai* obtained directly from Golden Hope Food & Beverages Sdn. Bhd (2008). The puree that was packed in an aseptic bag was stored immediately in freezer until the study was carried out (Golden Hope 2008). Once opened, the puree was repackaged into container of about 5 L for preparing the aqueous puree, at concentration of 500, 1000 and 2000 mg/kg body weight on low, medium and high dose group, dissolved in distilled water, respectively; every 3 days and stored again at -70ºC until used.

### EXPERIMENTAL PROCEDURE

Thirty male Sprague-Dawley rats each weighing between 200 and 280 g obtained from UKM animal house were kept one per metabolic cages in a temperature-controlled room at 25±2°C with a 12:12h light: darkness cycle with lights on at 8:00 am before starting the experiment. The rats were allowed free access to water and food during acclimatized week. The rats were divided into five groups: control negative (CN) fed with rat pellet; control positive, low, medium and high dose group (CP, LDG, MDG and HDG) were fed High Fat Diet-AIN93G, respectively. CN and CP were given distilled water; meanwhile treated group were given the aqueous puree, at concentration of 500, 1000 and 2000 mg/kg body weight, dissolved in distilled water were administered orally via a drinking bottle, respectively. All animals were observed daily for any clinical signs of disease. Body weight, blood chemistry and urine profile were measured through the study. After six weeks, the HFD induced-obese rats were fasted overnight (12-14 hours) and euthanized under an anesthetic condition with ethyl ether. Blood was collected from the posterior vena cava for biochemical

analysis on a blood haematology, enzyme activities, kidney function test and liver function test; respectively (Ayub et al. 2010). The organs were excised, weighed and immediately frozen in liquid nitrogen and stored at -70°C until further tests. The experiment was carried out at the MARDI (Malaysia Agriculture Research & Development Institute) animal house. The study was approved by the Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC).

### HIGH FAT DIET - AIN93G

The control negative (CN) was fed with the rat pellet diet, while the other four groups; control positive, low, medium and high dose group (CP, LDG, MDG and HDG) were given high fat fixed formulation diet based on AIN93G rat and mouse. The high fat content has resulted in a 74% increase in calculated energy. To allow for the high fat inclusion, the carbohydrate content has been reduced. The fatty acid profile has an increased proportion of saturated and monounsaturated fats. Changes in all other nutritional parameters have been kept to a minimum. The high fat content has necessitated a change in the diet form away from a pellet to a small block. The block contains around 12 g of diet and can be fed "as is" or cut into smaller sections for feeding. Table 1 shows the ingredients and nutritional parameters of the high fat diet - AIN93G.

### ANALYTICAL PROCEDURES

HFD induced-obese rats were fasted overnight (12-14 hours) and euthanized under an anesthetic condition using ethyl ether, after six weeks of oral administration. Blood was collected from the posterior vena cava, transferred into tube containing ethylene diamine tetraacetic acid (EDTA); and centrifuged at 3500 g for 20 min to obtain the plasma fraction. Serum was obtained by collecting blood in non-EDTA tube. The serum was used to determine kidney and liver function test. Plasma and serum samples were kept frozen at -70ºC until used. All analysis was done using Blood Chemical Analyzer (*Vitalab Selectra E, UK*) to measure the following parameters: blood hematology, enzyme activities, kidney and liver function test were calculated. Urine was collected after the rats were fasted overnight and analyzed by using a Urine Analyzer (*Bayer Diagnostics*) to measure the following parameters: glucose, bilirubin, ketones, specific gravity, blood, pH, protein, urobilinogen, nitrates and leukocyte esterase.

### STATISTICAL ANALYSIS

All data are presented as the mean ± standard error of mean (SEM). The data were evaluated by a one-way ANOVA using SAS System for Windows v6.12, and the differences between the means assessed using Duncan's multiple range test. Statistical significance was considered at $p<0.05$.

## RESULTS AND DISCUSSION

### EFFECT OF PINK GUAVA SUPPLEMENT ON BODY AND ORGANS WEIGHT

Pink guava (*Psidium guajava*) puree supplement had significantly decreased the body weight of HFD induced-obese rats. As shown in Table 2 the mean body weight was almost the same (~300 g) in all groups at the start of the study. At the time of killing, mean body weight was significantly lowest in HDG (413.70±37.22 g), followed by LDG (439.25±30.84 g) and MDG (444.94±39.01 g) compared to CN (447.00±32.76 g) and CP (467.24±47.77 g), respectively. Pink guava puree intake had effect on bodyweight gain. HFD-induced obese rats gained positive weight, indicating good health status.

TABLE 1. Ingredients and nutritional parameters of High Fat AIN93G Diet (HFD)

| Nutritional parameters | % | Ingredients | g/kg |
|---|---|---|---|
| Protein | 19.0 | Casein (Acid) | 200 |
| Total Fat | 60.0 | DL Methionine | 3.0 |
| Crude Fibre | 4.70 | Sucrose | 106 |
| Acid Detergent Fibre | 4.70 | Cellulose | 50 |
| Digestible Energy | 28 MJ/ kg | Canola Oil | 100 |
| | | Cocoa Butter | 400 |
| | | Clarified butter fat | 100 |
| | | Calcium Carbonate | 13.1 |
| | | Sodium Chloride | 2.6 |
| | | Potassium Citrate | 2.5 |
| | | Potassium Sulphate | 1.6 |
| | | AIN93G minerals | 1.4 |
| | | Choline Chloride | 2.5 |
| | | Potassium Dihydrogen Phosphate | 6.9 |

Organ's relative weight such as liver, heart, kidney, lung, spleen and testes (Table 2) were not affected by the pink guava puree supplementation. They were not significantly different compared to the CN and CP. Organ weight measurement is important to access general toxicity because any change in organ weight is a sensitive indicator of toxicity. This finding is similar to the Ayub et al. (2010) report. In theory, organ weight will be affected by the suppression of body weight as described by Marshall (2000). In this study, the pink guava puree supplement did not give any significant changes in the organs' relative weights of HFD induced-obese rats compared to control group.

Oral administration of pink guava puree drinking solution did not induce mortality up to the highest dose, which was 2000 mg/kg body weight. No HFD induced-obese rats showed any toxic signs such as nose bleeding, vomiting, 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 (OECD 2006). Thus, the result may suggest the pink guava puree dosage is more than 2000 mg/kg body weight. Norazmir et al. (2009) also reported similar results in sub-acute studies of pink guava puree in Spontaneous Hypertensive Rats.

### EFFECT OF PINK GUAVA SUPPLEMENT ON BLOOD HAEMATOLOGY

Blood hematology showed significant differences in HDG's red blood cell, hemoglobin and hematocrit amount (Table 3) compared to CN group. HDG's red blood cell count ($9.20\pm0.53 \times 10^{12}$/L) was significantly different compared to CN group ($10.78\pm1.38 \times 10^{12}$/L). HDG's hemoglobin (163.33±11.72 g/dL) was significantly different compared to CN group (192.17±26.42 g/dL). Hematocrit value also higher in HDG's (46.97±3.65) compared to CN group (55.55±7.87). This result was similar to Yin-Tzu (2008) study of guava extract on immune response. It showed that the action of the guava extract has increased the hematocrit value, but was not as efficient with carrying oxygen throughout the body, according to the hemoglobin value; vice-verse with this study result. The red blood cell indices suggested that

TABLE 2. Effects of pink guava puree on body and organ weights in High Fat Diet (HFD) induced-obese rats.

| Body weight (g) | Control Negative Group (pellet + water) | Control Positive Group (HFD + water) | Low Dose Group (HFD + 500 mg/kg bw) | Medium Dose Group (HFD + 1000 mg/kg bw) | High Dose Group (HFD + 2000 mg/kg bw) |
|---|---|---|---|---|---|
| Initial | 303.87±29.95$^{a}$ | 303.35±31.53$^{a}$ | 302.51±35.94$^{a}$ | 305.66±42.75$^{a}$ | 305.68±48.20$^{a}$ |
| Final | 447.00±32.76$^{a}$ | 467.24±47.77$^{a}$ | 439.25±30.84$^{b}$ | 444.94±39.01$^{b}$ | 413.70±37.22$^{b}$ |
| *Organ weight (g)* | | | | | |
| Liver | 15.02±2.66$^{a}$ | 12.66±0.54$^{ab}$ | 11.86±1.74$^{b}$ | 13.22±3.53$^{ab}$ | 11.20±0.82$^{b}$ |
| Heart | 1.17±0.16$^{a}$ | 1.18±0.12$^{a}$ | 1.10±0.09$^{a}$ | 1.17±0.16$^{a}$ | 1.18±0.22$^{a}$ |
| Kidney | 2.55±0.23$^{a}$ | 2.51±0.28$^{a}$ | 2.44±0.22$^{a}$ | 2.53±0.25$^{a}$ | 2.30±0.28$^{a}$ |
| Lung | 1.91±0.29$^{a}$ | 1.97±0.04$^{a}$ | 1.82±0.23$^{a}$ | 2.00±0.52$^{a}$ | 1.97±0.27$^{a}$ |
| Spleen | 0.69±0.09$^{a}$ | 0.61±0.07$^{a}$ | 0.70±0.29$^{a}$ | 0.63±0.08$^{a}$ | 0.70±0.16$^{a}$ |
| Testes | 3.47±0.13$^{ab}$ | 3.08±0.21$^{ab}$ | 3.18±0.25$^{ab}$ | 3.75±0.80$^{a}$ | 2.75±1.36$^{b}$ |

Means with the same letter in same row are not significantly different ($p<0.05$); n=6

TABLE 3. Blood hematology of High Fat Diet (HFD) induce-obese rats supplemented with pink guava puree

| | Control Negative Group (pellet + water) | Control Positive Group (HFD + water) | Low Dose Group (HFD + 500 mg/kg bw) | Medium Dose Group (HFD + 1000 mg/kg bw) | High Dose Group (HFD + 2000 mg/kg bw) |
|---|---|---|---|---|---|
| RBC | 10.78±1.38$^{a}$ | 9.36±1.39$^{ab}$ | 9.40±0.68$^{ab}$ | 9.55±1.28$^{ab}$ | 9.20±0.53$^{b}$ |
| WBC | 7.42±1.69$^{a}$ | 4.70±1.60$^{b}$ | 4.42±1.19$^{b}$ | 5.58±1.48$^{ab}$ | 5.53±3.21$^{ab}$ |
| PLT | 1350.8±163.8$^{a}$ | 1089.5±127.0$^{b}$ | 1092.6±213.3$^{b}$ | 1190.3±251.1$^{ab}$ | 1260.0±138.7$^{ab}$ |
| Hb | 192.17±26.42$^{a}$ | 166.83±20.51$^{ab}$ | 167.00±11.08$^{ab}$ | 168.00±24.22$^{ab}$ | 163.33±11.72$^{b}$ |
| HCT | 55.55±7.87$^{a}$ | 48.95±6.57$^{ab}$ | 48.55±2.86$^{ab}$ | 47.87±7.71$^{ab}$ | 46.97±3.65$^{b}$ |

Means with the same letter in same row are not significantly different ($p<0.05$); n=6

*Note*:

RBC : Red Blood Cell ($10^{12}$/L)
WBC : White Blood Cell ($10^{9}$/L)
PLT : Platelet ($10^{9}$/L)
Hb : Haemoglobin (g/dL)
HCT : Hematocrit (%)

the *Psidium guava* extract has no adverse effect on the HFD-induced obese rats.

### EFFECT OF PINK GUAVA SUPPLEMENT ON ANTIOXIDANT ENZYME ACTIVITIES

The specific activities of glutathione peroxidase (GPx), glutathione reductase (GR) and superoxide dismutase (SOD) and total antioxidant status (TAS) concentration are given in Table 4. GPx, GR and SOD specific activities of the HFD-induced obese rats were significantly increased compared to the CN rats. Specific activity for GPx was significantly higher in HDG (2897.33±674.97 U/L), MDG (2819.50±262.04 U/L) and LDG (2787.50±266.36 U/L) compared to CN (2184.50±816.59 U/L) and CP (2610.17±61.63 U/L), respectively. Specific activity for SOD also significantly higher in HDG (418.67±35.48 U/L), MDG (409.33±55.22 U/L) and LDG (404.67±18.32 U/L) compared to CN (164.33±43.81 U/L) and CP (341.33±60.27 U/L), respectively. GR specific activity was significantly different in HDG (203.00±10.30 U/L) and MDG (181.00±30.26 U/L) compared to CN (116.17±10.76 U/L).

Administering pink guava puree to the HFD-induced obese rats significantly increased those antioxidant enzyme activities. The effect was more pronounced in the HDG supplemented group than in the CN or CP group. In a rat model of diet-induced obesity, Dobrian et al. (2000) reported increase in the activities of erythrocyte CuZn-SOD and GPx after 10 weeks on the diet. It attributed the increase in SOD and GPx enzymes, which are antioxidants, to their stimulation by oxidative stress. Similarly, Vincent et al. (1999) study of obese Zucker rats, reported increased activities of SOD and GPX. The similarity between our results and those studies of Dobrian et al. (2000) and Vincent et al. (1999) could be due to the duration of the obesity. It is likely that, in the early days of the development of obesity, antioxidant enzyme activity will be stimulated. However, once the obesity persists for a long time, as in humans, the sources of the antioxidant enzymes become depleted, leading to a low level of activity, as we found in total antioxidant status. Total antioxidant status of the treated groups did not show significant differences compared to the CN and CP group. Prince and Menon (1999) study 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 SUPPLEMENT ON KIDNEY FUNCTION TEST

Kidney function test of urea concentration were significantly decreased in HFD-induced obese rats; LDG (4.28±0.69 mmol/L), MDG (4.35±0.87 mmol/L) and HDG (3.85±0.71 mmol/L) as compared to CN (7.02±1.81 mmol/L) respectively as shown in Table 5. Creatinine and uric acid concentrations did not show any significant differences between supplemented pink guava-treated rats compared to CN and CP group. Creatinine value for treated group ranged from 71.98-78.90 μmol/L compared to control group ranged from 75.01-75.17 μmol/L; meanwhile uric acid value ranged from 0.33-0.41 mmol/L.

TABLE 4. Effect of pink guava puree on enzyme activities in High Fat Diet (HFD) induce-obese rats

| | Control Negative Group (pellet + water) | Control Positive Group (HFD + water) | Low Dose Group (HFD + 500 mg/ kg bw) | Medium Dose Group (HFD + 1000 mg/ kg bw) | High Dose Group (HFD + 2000 mg/ kg bw) |
|---|---|---|---|---|---|
| GPx | 2184.5±816.6$^{b}$ | 2610.2±61.6$^{b}$ | 2787.5±266.4$^{a}$ | 2819.5±262.0$^{a}$ | 2897.3±674.9$^{a}$ |
| SOD | 164.33±43.81$^{c}$ | 341.33±60.27$^{b}$ | 404.67±18.32$^{a}$ | 409.33±55.22$^{a}$ | 418.67±35.48$^{a}$ |
| GR | 116.17±10.76$^{c}$ | 132.50±19.41$^{bc}$ | 137.33±9.69$^{bc}$ | 181.00±30.26$^{ab}$ | 203.00±10.30$^{a}$ |
| TAS | 1.33±0.19$^{a}$ | 1.35±0.14$^{a}$ | 1.44±0.22$^{a}$ | 1.56±0.29$^{a}$ | 1.63±0.63$^{a}$ |

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

*Note:*

GPx : Glutathione peroxidase (U/L)
SOD : Superoxide dismutase (U/L)
GR : Glutathione reductase (U/L)
TAS : Total antioxidant status (mmol/L)

TABLE 5. Kidney function test of High Fat Diet (HFD) induce-obese rats supplemented with pink guava puree

| | Control Negative Group (pellet + water) | Control Positive Group (HFD + water) | Low Dose Group (HFD + 500 mg/ kg bw) | Medium Dose Group (HFD + 1000 mg/ kg bw) | High Dose Group (HFD + 2000 mg/ kg bw) |
|---|---|---|---|---|---|
| Creatinine(μmol/L) | 75.01±3.83$^{a}$ | 75.17±6.35$^{a}$ | 77.38±7.78$^{a}$ | 78.90±12.20$^{a}$ | 71.98±7.91$^{a}$ |
| Urea (mmol/L) | 7.02±1.81$^{a}$ | 3.92±0.49$^{b}$ | 4.28±0.69$^{b}$ | 4.35±0.87$^{b}$ | 3.85±0.71$^{b}$ |
| Uric acid (mmol/L) | 0.38±0.24$^{a}$ | 0.41±0.19$^{a}$ | 137.33±9.69$^{bc}$ | 181.00±30.26$^{ab}$ | 203.00±10.30$^{a}$ |

Means with the same letter in same row are not significantly different (p<0.05); n=6

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). Plasma urea and creatinine concentrations are often used as an index of renal glomerular function and will be increased in renal injuries (Hughes & Jefferson 2008). 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). Creatinine is a nitrogenous waste product produced from creatinine in muscle and excreted by the kidneys. The majority of creatinine is excreted by glomerular filtration, but a small portion (~10%) is secreted into the proximal tubular lumen. The normal serum concentration of creatinine varies considerably between 60-120 μmol/L, depending on muscle mass, and can be used to estimate renal function. Nzi et al. 2007 found that based on biochemical analysis of renal and hepatobiliary functions, such as the level of urea, creatinine and alkaline phosphate value, the fruit extract/ juices generally tolerated by rats. These findings were similar with this study.

### EFFECT OF PINK GUAVA SUPPLEMENT ON LIVER FUNCTION TEST

The activities of total protein, albumin, globulin, AG ratio, total bilirubin, ALP, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) level of treated group and control group are given in Table 6. Pink guava supplement showed significantly decreased levels of total protein, globulin and ALT for treated group as compared to CN group. Total protein of MDG (72.67±3.65 g/L) and HDG (76.00±2.49 g/L) were significantly lower compared to CN (80.11±1.98 g/L). Globulin value for LDG (34.17±3.43 g/L), MDG (32.17±1.83 g/L) and HDG (35.00±3.41 g/L) were significantly lower compared to CN (39.67±0.82 g/L). AG ratio for LDG (1.22±0.16), MDG (1.28±0.07) and HDG (1.19±0.14) significantly different compared to CN (1.03±0.08). ALT value were also significantly lower for LDG (55.83±15.12 U/L), MDG (50.67±22.65 U/L) and HDG (57.50±8.48 U/L) compared to CN (77.00±16.26 U/L), respectively.

Liver is the target organ because most toxicants enter the body via the gastrointestinal tract, and after absorption, the toxicants are carried by the hepatic portal vein to the liver. These parameters are commonly used to evaluate the status of liver function (Norazmir & Ayub 2010). Liver function test is crucial because liver is the central organ in detoxification of compounds. 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, globulin and ALT concentrations were not affected by the pink guava puree in treated group compared to CN. This shows that the synthesis of protein in the HFD induced-obese rat's liver is not influenced by the supplementation. Similar results were also obtained in the studies of *Psidium guajava* on Spontaneous Hypertensive Rats (Norazmir & Ayub 2009). 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).

### EFFECT OF PINK GUAVA SUPPLEMENT ON URINE PROFILE

Table 7 showed the urine profile between dosage groups on the last day of experiment. No glucose and blood were

TABLE 6. Liver function test of High Fat Diet (HFD) induce-obese rats supplemented with pink guava puree

| | Control Negative Group (pellet + water) | Control Positive Group (HFD + water) | Low Dose Group (HFD + 500 mg/ kg bw) | Medium Dose Group (HFD + 1000 mg/ kg bw) | High Dose Group (HFD + 2000 mg/ kg bw) |
|---|---|---|---|---|---|
| Protein (g/L) | 80.11±1.98$^{a}$ | 75.02±3.88$^{b}$ | 76.26±3.86$^{ab}$ | 72.67±3.65$^{b}$ | 76.00±2.49$^{b}$ |
| Albumin (g/L) | 40.60±2.58$^{a}$ | 40.90±2.22$^{a}$ | 41.78±2.63$^{a}$ | 40.85±2.61$^{a}$ | 41.15±1.52$^{a}$ |
| Globulin (g/L) | 39.67±0.82$^{a}$ | 34.17±2.32$^{b}$ | 34.17±3.43$^{b}$ | 32.17±1.83$^{b}$ | 35.00±3.41$^{b}$ |
| AG ratio | 1.03±0.08$^{b}$ | 1.20±0.08$^{a}$ | 1.22±0.16$^{a}$ | 1.28±0.07$^{a}$ | 1.19±0.14$^{a}$ |
| Bilirubin($\mu$mol/L) | 6.18±1.05$^{a}$ | 4.67±2.12$^{a}$ | 4.99±2.01$^{a}$ | 4.63±0.86$^{a}$ | 4.65±0.76$^{a}$ |
| ALP (U/L) | 122.00±30.62$^{a}$ | 123.50±39.83$^{a}$ | 96.50±43.13$^{a}$ | 114.17±35.27$^{a}$ | 104.83±29.44$^{a}$ |
| ALT (U/L) | 77.00±16.26$^{a}$ | 53.67±11.09$^{b}$ | 55.83±15.12$^{b}$ | 50.67±22.65$^{b}$ | 57.50±8.48$^{b}$ |
| AST (U/L) | 132.67±29.62$^{a}$ | 93.33±21.80$^{a}$ | 102.33±12.55$^{a}$ | 132.33±39.00$^{a}$ | 104.33±16.18a$^{a}$ |

Means with the same letter in same row are not significantly different ($p<0.05$); n=6

*Note:*

ALP : Alkaline phosphate
ALT : Alanine aminotransferase
AST : Aspartate aminotransferase

TABLE 7. Urine profile of High Fat Diet (HFD) induce-obese rats supplemented with pink guava puree

| Urine profile components | Control Negative Group (pellet + water) | Control Positive Group (HFD + water) | Low Dose Group (HFD + 500 mg/ kg bw) | Medium Dose Group (HFD + 1000 mg/kg bw) | High Dose Group (HFD+ 2000 mg/ kg bw) |
|---|---|---|---|---|---|
| Specific gravity | 1.005[a] | 1.015[a] | 1.015[a] | 1.015[a] | 1.005[a] |
| pH | 6.9[a] | 6.8[a] | 7.3[a] | 7.3[a] | 7.7[a] |
| Ketones | -ve | Trace | Trace | Trace | -ve |
| Blood | 1.5 | -ve | -ve | -ve | -ve |
| Protein | Trace | 1.7 | 2 | 1.7 | 1.3 |
| Nitrates | -ve | Trace | -ve | -ve | -ve |
| Glucose | -ve | -ve | -ve | -ve | -ve |
| Urobilinogen (μmol/L) | Normal | Normal | Normal | Normal | Normal |
| Leukocyte | -ve | -ve | -ve | -ve | -ve |

Means with the same letter in same row are not significantly different ($p<0.05$); n=6

found in the urine of supplemented groups. The values of urobilinogen, bilirubin, nitrates and leukocyte esterase in HDG, MDG and LDG same as the CP and CN values. Glucose and bilirubin can be found in urine when the kidneys are damaged or diseased (Hughes & Jefferson 2008). Nitrites were present in the CP group. Bacteria that cause a urinary tract infection (UTI) have an enzyme that can convert urinary nitrates to nitrites (Lahlou et al. 2006). Therefore, nitrites in urine are an indication of UTI. Human trial was also conducted with *Hibiscus sabdariffa* calyces (Herrera-Arellano et al. 2004). Hypertensive patients were recruited and aged between 30 and 80 years of age. *Hibiscus sabdariffa* (10 g) was consumed with 0.5 L of water at breakfast for 4 weeks. Results showed that pH values showed no significant differences by *Hibiscus sabdariffa, which* is similar with Norazmir et al. (2009) study using pink guava puree on Spontaneous Hypertensive Rats.

## CONCLUSION

In conclusion, this study demonstrated that pink guava (*Psidium guajava*) puree supplement showed no toxicity effect. Pink guava puree supplements seem to be beneficial for increased antioxidant enzyme activities; with significant result to reduce body weight, blood hematology, kidney and liver function test showed extensively differences in treated group compared to control group; and correcting/ preventing obesity complications in kidney and liver functions.

## ACKNOWLEDGEMENTS

This research was supported financially by Universiti Kebangsaan Malaysia through the grant UKM-ABI-NBD00011-2007 and UKM-GUP-BTK-08-14-307. The authors gratefully acknowledge Golden Hope Food & Beverages Sdn. Bhd., Cucu Cahyana, Syahida Maarof, Norlia Jainal (staff of School of Chemical Sciences & Food Technology, Universiti Kebangsaan Malaysia), Hadijah Hassan, Ahmad Tarmizi Salimin and Wan Abd Aziz (Malaysia Research & Development Institute) for technical assistance.

## REFERENCES

Appel, L.J., Moore, T.J., Obarzanek, E., Vollmet, W.M., Svetkey, L.P., Sacks, F.M., Bray, G.A., Vogt, T.M., Cutler, J.A., Windhauser, M.M., Lin, P.H. & Karanja, N. 1997. A clinical trial of the effects of dietary patterns on blood pressure. *New England Journal of Medicine* 336: 1117-1124.

Asmah, R., Mohd, F.A.B. & Zarida, H. 2006. The effects of guava (Psidium guava) consumption on total antioxidant and lipid profile in normal male youth. *African Journal of Food Agriculture Nutrition and Development* 6(2): 1-12.

Ayub, M.Y., Norazmir, M.N., Mamot, S., Jeeven, K. & Hadijah, H. 2010. Anti-hypertensive effect of pink guava (Psidium guajava) puree on Spontaneous Hypertensive Rats. *International Food Research Journal* 79: 89-96.

Cerutti, P.A. 1994. Oxy-radicals and cancer. *Lancet* 344: 796-798.

Cookson, M. & Shaw, P. 1999. Oxidative stress and motor neuron disease. *Brain Pathology* 9(1): 165-86.

Datta, S., Sinha, S. & Bhattacharyya, P. 1999. Effect of an herbal protein, CI-I, purified from Cajanus indicus, in models of liver failure in mice. *Drug Development Research* 48: 76-83.

Davì, G., Falco, A. & Patrono, C. 2005. Lipid peroxidation in diabetes mellitus. *Antioxidant Redox Signal* 7(1-2): 256-68.

Dobrian, A.D., Davies, M.J., Prewitt, R.L. & Lauterio, T.J. 2000. Development of hypertension in a rat model of diet-induced obesity. *Hypertension* 35: 1009-1015.

Golden Hope Food and Beverages Sdn. Bhd., 2008. Pink guava puree processing. In *Lycopene content and lipophilic antioxidant capacity of by-products from Psidium guajava fruits produced during puree production industry*. *Food Bioproducts Processing,* edited by Kong, K.W. & Ismail, A. 2010. doi:10.1016/j.fbp.2010.02.004

Herrera-Arellano, A., Flores-Romero, S., Chavez-Soto, M. & Tortoriello, J. 2004. Effectiveness and tolerability of a standardized extract from *Hibiscus sabdariffa* in patients with mild to moderate hypertension: A controlled and randomized clinical trial. *Phytomedicine* 11: 375-382.

Hitchon, C. & El-Gabalawy, H. 2004. Oxidation in rheumatoid arthritis. *Arthritis Research & Therapy* 6(6): 265–78.

Hughes, J. & Jefferson, A. 2008. *Clinical Chemistry*. Churchill Livingstone: Elsevier.

James, P.T. 2004. Obesity: The worldwide epidemic. *Clinical Dermatology* 22: 276-280.

Kadiri, S., Arije, A. & Salako, B.L. 1999. Tradititional herbal preparations and acute renal failure in South West Nigeria. *Journal of Tropical Doctor* 29(4): 244-246.

Lahlou, S., Tahraoui, A., Israili, Z. & Lyoussi, B. 2006. Diuretic activity of the aqueous extracts of *Carum carvi* and *Tanacetum vulgare* in normal rats. *Journal of Ethnopharmacology* 110(3): 458-463.

Latha, M., Ananthan, K.R., Ramkumar, M., Pari, L., Baskar, C. & Narmatha, V. 2003. Effect of *Gymnema montanum* leaves on serum and tissue lipids in alloxan diabetic rats. *Experimental Diabetic Research* 4(3): 183-189.

Marshall, W.J. 2000. *Clinical Chemistry*. 4th ed. Edinburgh: Mosby.

Moshi, M.J., Lutale, J.J.K., Rimoy, G.H., Abbas, Z.G., Josiah, R.M. & Andrew, B.M. 2001. The effect of *Phyllanthus amarus* aqueous extract on blood glucose in non-insulin dependent diabetic patients. *Research Journal of Phytochemistry* 15: 577-580.

Norazmir, M.N., M.Y. Ayub & M.M.A. Ummi. 2010. Enzyme activities and histology study on high fat diet-induced obese rats by pink guava puree. *Pakistan Journal of Nutrition* 9(11): 1100-1106.

Norazmir, M.N. & M.Y. Ayub, 2010. Beneficial lipid-lowering effects of pink guava puree in high fat diet induced-obese rats. *Malaysian Journal of Nutrition* 16(1): 171-185.

Norazmir, M.N., Ayub, M.Y. & Mamot, S. 2009. Blood and urine profiles of Spontaneous Hypertensive Rats supplemented with pink guava (*Psidium guajava*) puree. *Sains Malaysiana* 38(6): 929-934

Norazmir, M.N., Ayub, M.Y., Mamot, S., Hadijah, H. & Ahmad Tarmizi, S. 2009. Effects of pink guava (*Psidium guajava*) puree supplementation on Spontaneous Hypertensive Rat's enzyme activities. *Proceedings of the 2nd UKM - Universitas Indonesia Seminar*. p. 75. Bangi: Universiti Kebangsaan Malaysia.

Nunomura, A., Castellani, R., Zhu, X., Moreira, P., Perry, G. & Smith, M. 2006. Involvement of oxidative stress in Alzheimer disease. *Journal of Neuropathology Exp Neurology* 65(7): 631-41.

Nzi, A.K., Elfriede, M.B., Nilton, L., Soraya, D. & Varela, E.A. 2007. Acute, sub-acute toxicity and genotoxic effect of a hydroethanolic extract of the cashew (*Anacardium occidentale* L.). *Journal of Ethnopharmacology* 110: 30-38.

OECD. 2006. Draft Final Report of the Validation of the Updated Test Guideline 407 Repeat Dose 28-day Oral Toxicity Study in Laboratory Rats.

Olusi, S.O. 2002. Obesity is an independent risk factor for plasma lipid peroxidation and depletion of erythrocyte cytoprotectic enzymes in humans. *International Journal of Obesity* 26: 1159-1164.

Prince, P.S. & Menon, V.P. 1999. Antioxidant activity of Tinospora cordifolia roots in experimental diabetes. *Journal of Ethnopharmacology* 65: 277-281.

Russo, C., Oliviri, O., Girelli, D., Faccini, G., Zenari, M.L., Lombardi, S. & Corrocher, R. 1998. Anti-oxidant status and lipid peroxidation in patients with essential hypertension. *Journal of Hypertension* 16: 1267-1271.

Steinberg, D. 1997. A critical look at the evidence for the oxidation of LDL in atherogenesis. *Atherosclerosis* 13: (Suppl) 5-7.

Thaipong, K., Unaroj, B., Kevin, C., Luis, C.Z. & David, H.B. 2006. Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis* 19: 669-675.

Vaughn, G. 1999. *Understanding and Evaluating Common Laboratory Test*. Stamford: Appleton & Lange.

Vincent, H.K., Powers, S.K., Stewart, D.J., Shanely, R.A., Demirel, H. & Nalto, H. 1999. Obesity is associated with increased myocardial oxidative stress. *International Journal of Obesity Related Metabolic Disorder* 23: 67-74.

Wiseman, H. & Halliwell, B. 1996. Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. *Biochemistry Journal* 313: 17-29.

Wood-Kaczmar, A., Gandhi, S. & Wood, N. 2006. Understanding the molecular causes of Parkinson's disease. *Trends Molecular Medicine* 12(11): 521-528.

Yin, T.L. 2008. Influence of guava (*Psidium guajava*) extract on immune response of *Puntius altus*. Tesis Sarjana Muda. Unpublished. Mahidol University International College Thailand.

Ayub Mohd Yatim
(Deceased)

Norazmir Md Nor*
Department of Nutrition and Dietetics
Faculty of Health Sciences
MARA University of Technology
Puncak Alam Campus
42300 Puncak Alam
Selangor D.E.
Malaysia

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

Received: 4 December 2009
Accepted: 15 July 2010