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Beneficial Lipid-Lowering Effects of Pink Guava Puree in High Fat Diet Induced-Obese Rats

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ABSTRACT

A study was carried out to determine the anti-obesity effects of pink guava (*Psidium guajava*) puree in high fat diet (HFD) induced-obese rats. Thirty male Sprague-Dawley rats were divided into 5 groups: control negative (CN), fed normal rat pellet; control positive (CP), low, medium and high dosage group (LDG, MDG, HDG) were fed HFD, respectively. CN and CP groups were given distilled water; meanwhile treated groups were given the aqueous puree dissolved in distilled water administered orally for six weeks. The results obtained showed that pink guava puree significantly decreased the body weight and systolic blood pressure of HFD induced-obese rats as compared to control. Blood glucose values for treated groups (4.3-4.9 mmol/L) were significantly lower as compared to CN and CP (5.7 and 5.8 mmol/L) respectively. HDG showed a significant reduction in 34.47% total cholesterol (TC) levels followed by MDG (23.30%) and LDG (22.33%). Triglycerides (TG) levels for all treated groups especially HDG (43.59%) showed significant difference as compared to control. High density lipoprotein-cholesterol (HDL-C) levels showed an increase in the treated group as compared to control. Low density lipoprotein-cholesterol (LDL-C) levels significantly decreased in HDG (69.70%), MDG (39.40%) and LDG (37.12%) as compared to control. Kidney function tests showed significant changes in urea concentrations in treated groups as compared to control. Liver function tests showed significant differences in globulin, A:G ratio, alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and CK-Nac in treated groups as compared to control. Specific enzyme activities of glutathione peroxidase (GPx) was significantly higher in LDG (2787.50±266.36 U/L), MDG (2819.50±262.04 U/L) and HDG (2897.33±674.97 U/L) respectively, as compared to CN (2184.50±816.59 U/L) and CP (2610.17±61.63 U/L). Significant differences were also seen in superoxidase dismutase (SOD) activities in treated groups as compared to control. In conclusion, this study found that pink guava puree had anti-obesity properties and high enzyme activities.

Keywords: Obesity, pink guava, high fat diet-induced rats

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INTRODUCTION

Obesity has increased at an alarming rate and is now a worldwide health problem. It is known that obesity results from disequilibrium between energy intake and expenditure, and obesity is known to be a strong risk factor for Type 2 diabetes associated with insulin resistance (Bray *et al.*, 2002). Obesity is the most common nutritional disorder in the developed world and it is considered a risk factor associated with the development of major human diseases, including cardiovascular disease, diabetes, and cancer. A daily consumption of diets high in fat tends to promote obesity. Increased intake of high caloric (energy and fat) food promotes body fat storage and greater body weight and adiposity in humans (Bray *et al.*, 2002) and animals (Estadella *et al.*, 2004). Over-the-counter remedies based on nutritional supplements are extremely popular, especially with respect to obesity and body composition. Inhibition of the digestion and absorption of dietary fat has been used as targets in obesity treatment (Weigle, 2003).

Guava (*Psidium guajava*) is widely cultivated and popular; commonly known as either guava, guyava and kuawa, it is thought to be a useful medicinal plant. The place of origin of the guava is uncertain but propagation of the fruits needs a tropical location and sunlight. Previous studies of guava properties demonstrated it contains anti-diabetic and anti-inflammatory properties (Mukherjee, Saha & Saha, 1997), anti-diarrhea properties (Lutterodt, 1989), bio-anti-mutagenic properties (Matsuo *et al.*, 1994), anti-pyretic properties (Shinha *et al.*, 2000), anti-microbial properties (Jaiarj *et al.*, 1999), hepato-protective properties (Sohn *et al.*, 2003), anti-oxidant and anti-hypertensive properties (Ayub *et al.*, 2010).

Red-fleshed Brazilian guava has several carotenoids such as phytofluene, ß-carotene, ß-cryptoxanthin, lycopene, rubixanthin, and lutein (Thaipong *et al.*, 2006). An alcohol extract of guava leaves exhibited spasmolytic effects on isolated rat and guinea-pig ileum and also effects of guava leaves on acute diarrheic disease has been described (Lozoya *et al.*, 2002). Leaves of this plant have been reported to contain several compounds such as various terpenoids (Begum *et al.*, 2002) and tannins.

However, in spite of its usefulness for the treatment of obesity, the mechanism of anti-obesity action remains to be resolved. Previous studies on the effect of cholesterol-rich diet on the development of atherosclerosis in rabbits have shown that atherosclerosis can be easily induced by feeding rabbits with 1% to 2% cholesterol. The most convincing evidence is the fact that, in animal species, atherosclerosis may be experimentally induced by the use of a high fat diet (Wybraniec *et al.*, 2001). There are no reports on the aspects of health potential of this fruit puree, despite the many claims that have been made. Therefore, the study aims to determine the effects of locally grown pink guava (*Psidum guajava*) puree on body weight, blood pressure, lipid profile, biochemistry profile and antioxidative enzyme activities in high fat diet induced-obese rats.

MATERIAL AND METHODS

Pink guava puree sample

Pink guava (*Psidium guajava*) puree, from the variety *Beaumont Sungkai* and *Beaumont Semenyih,* were obtained from a food & beverages company. Ayub *et al.* (2010) have studied the contents of the pink guava puree. The puree that was packed in an aseptic bag was stored immediately at -70ºC until further use. On opening, the puree was repackaged into a 5L container to make an aqueous puree, at concentrations of 500, 1000 and 2000 mg/kg body weight on low (5%), medium (10%) and high (20%) dosages, dissolved in distilled water respectively, every 3 days, and stored again at -70ºC until further use.

Experimental procedure

A total of thirty (30) healthy male Sprague Dawley (SD) rats each weighing between 250-280 g obtained from UKM Animal House were kept one per individual cage in a temperature-controlled room at 25±2°C with a 12:12h light:darkness cycle with lights on at 7:00 am before starting the experiment. All SD rats were acclimatised for a week at the beginning of the treatments. The animals were allowed free access to water and food. The rats were divided into 5 groups: control negative (CN) fed with rat pellet; control positive (CP), low, medium and high dosage groups (LDG, MDG, HDG) were fed HFD (AIN93G-purified rodent diet), respectively. CN and CP groups were given distilled water; meanwhile treated groups were given the aqueous puree dissolved in distilled water, at a concentration of 500, 1000 and 2000 mg/kg and administered orally via a drinking bottle. Body weight, water and food consumption, systolic blood pressure and blood chemistry were measured throughout the study. After 6 weeks, the HFD induced-obese rats were fasted overnight (12-14 hours) and euthanised under an anesthetic condition with diethyl ether. Blood was collected from the posterior vena cava for biochemical analysis, lipid profile, kidney function test, liver function test and enzyme activities. Blood from the tail was taken to measure glucose level. Organs such as liver, heart, kidney, lung, spleen and testes were excised, weighed and immediately frozen in liquid nitrogen and stored at -70°C until further tests. The study was approved by the Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC).

Organ relative weight

The liver, heart, kidney, lung, spleen and testes were quickly excised, rinsed in 0.9% cold saline to remove blood, blotted dry and immediately soaked in liquid nitrogen. The organ relative weight (percentage body weight) was obtained by dividing the final weight of organ to the final body weight (Sachan & Yatim, 1992).

High fat diet (HFD) AIN93G induced-obesity in rats

The control negative (CN) was fed with rat pellet diet, while four other groups, control positive (CP), low, medium and high dosage group (LDG, MDG and HDG), were given high fat fixed formulation diet based on AIN93G rat and mouse. The high fat content resulted in a 74% increase in calculated energy. To allow for the high fat inclusion, the carbohydrate content had been reduced. The fatty acid profile has an increased proportion of saturated and mono-unsaturated fats. Changes in all other nutritional parameters have been kept to a minimum. In one research facility, this formulation induced clear evidence of insulin resistance after six weeks of feeding. The high fat content necessitated a change in the diet form away from a pellet to a small block. The block contains around 12 grams of diet and can be fed 'as is' or cut into smaller sections for feeding. The nutritional composition of the rat pellet diet and high fat AIN93G diet is presented in Table 1.

Measurement of systolic blood pressure (SBP)

Systolic blood pressure was measured by using the tail-cuff method (IITC Life Science, Model 179 Non-Invasive Blood Pressure (NIBP) Multi Channel Blood Pressure System, USA). Blood pressure was taken with the rats under conscious conditions at the beginning of the experiment and weekly. Systolic blood pressure values were the means of three measurements per rat. During acclimatisation, the rats were placed in the restrainers for 10 to 15 minutes at 30°C daily to ensure the rats were comfortable with the restrainers, tail-cuff detector and warming chamber.

Table 1. Calculated nutritional parameter and high fat AIN93G ingredients

Calculated nutritional parameters	*%*
Protein	19.0
Total fat	60.0
Crude fibre	4.70
Acid detergent fibre	4.70
Digestible energy	28 MJ / Kg
Ingredients	*g/kg*
Casein (Acid)	200
DL Methionine	3.0
Sucrose	106
Cellulose	50
Canola oil	100
Cocoa butter	400
Clarified butter fat (Ghee)	100
Calcium Carbonate	13.1
Sodium Chloride	2.6
Potassium Citrate	2.5
Potassium Dihydrogen Phosphate	6.9
Potassium Sulphate	1.6
AIN93G Trace minerals	1.4
Choline Chloride (65%)	2.5
AIN93G Vitamins	10

Source: *Specialty Feeds*, Australia

Analytical procedures

After six weeks of oral administration, HFD induced-obesed rats were fasted overnight (12-14 hours) and euthanised under an anesthetic condition using diethyl ether. Tail blood was taken by clipping for glucose determination. Blood was collected from the posterior vena cava, transferred into a tube containing ethylene diamine tetraacetic acid (EDTA), and centrifuged at 3500 g for 20 minutes to obtain the plasma fraction. The plasma samples were kept frozen at -70°C until further use, while the whole blood was used to measure glutathione peroxidase, superoxide dismutase and glutathione reductase levels. Serum was obtained by collecting blood in a non-EDTA tube. The serum was used to determine kidney and liver function test. Plasma and serum samples were kept at -70ºC. All analysis was done using Blood Chemical Analyser (*Vitalab Selectra E, UK*) to measure the following parameters: biochemical analysis, lipid profile, kidney function test, liver function test and antioxidative enzyme activities.

Statistical analysis

The significant differences between the control and treated groups were analysed using Duncan's Multiple Range Test. All mean values are expressed as group means ± standard error of mean (SEM). The minimal level of significance accepted was $p<0.05$.

RESULTS AND DISCUSSION

Effect of pink guava puree on body and organ weights

Supplementation of pink guava puree significantly decreased the body weights of HFD induced-obese rats. As shown in Figure 1, mean body weights were almost similar (~300 g) in all groups at the beginning of the study. At the time of killing, mean body

Table 2. Effects of pink guava puree on body and percentage of organ weights in HFD induced-obese rats

Body weight (g)	*CN*	*CP*	*LDG*	*MDG*	*HDG*
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 weight	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}
% body weight	3.37±0.60^{a}	2.72±0.12^{b}	2.73±0.39^{b}	2.97±0.78^{ab}	2.72±0.19^{b}
Heart weight	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}
% body weight	0.27±0.05^{a}	0.27±0.05^{a}	0.23±0.05^{a}	0.27±0.05^{a}	0.30±0.06^{a}
Kidney weight	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}
% body weight	0.58±0.04^{a}	0.53±0.05^{a}	0.55±0.05^{a}	0.57±0.08^{a}	0.55±0.08^{a}
Lung weight	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}
% body weight	0.43±0.05^{ab}	0.40±0.01^{b}	0.43±0.05^{ab}	0.43±0.10^{ab}	0.50±0.06^{a}
Spleen weight	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}
% body weight	0.17±0.05^{a}	0.12±0.04^{a}	0.13±0.08^{a}	0.13±0.05^{a}	0.17±0.05^{a}
Testes weight	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}
% body weight	0.77±0.05^{a}	0.67±0.05^{a}	0.72±0.08^{a}	0.68±0.33^{a}	0.77±0.05^{a}

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

Note
CN : Control Negative (rat pellet + water)
CP : Control Positive (HFD + water)
LDG : Low Dosage Group (HFD + 5% pink guava puree)
MDG : Medium Dosage Group (HFD + 10% pink guava puree)
HDG : High Dosage Group (HFD + 20% pink guava puree)
HFD : High Fat Diet AIN93G

weights were significantly lowest in HDG (413.70 ± 37.22 g), followed by LDG (439.25 ± 30.84 g) and MDG (444.94 ± 39.01 g) as compared to CN (447.00 ± 32.76 g) and CP (467.24 ± 47.77 g), respectively. Pink guava puree intake has a beneficial effect on lowering bodyweight. This finding is similar to Nzi *et al*. (2007).

All HFD induced-obesed rats gained weight, indicating good health status. 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 as compared to CN and CP groups. Organ weight measurement is important to access general toxicity because any change in organ weight is a sensitive indicator of toxicity. 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. In theory, organ weight will be affected by the suppression of body weight as described by Hadijah *et al.* (2004). In this study, the pink guava puree supplementation did not result in any significant changes in the relative weights of organs of HFD induced-obese rats as compared to control groups.

Oral administration of pink guava (*Psidium guajava*) puree drinking solution did not induce mortality up to the highest dose, which was 2000 mg / kg body weight. No HFD induced-obesed rats showed any toxic signs such as nose bleeding, vomiting, fur loss, diarrhea and death throughout the observation period. The administration of

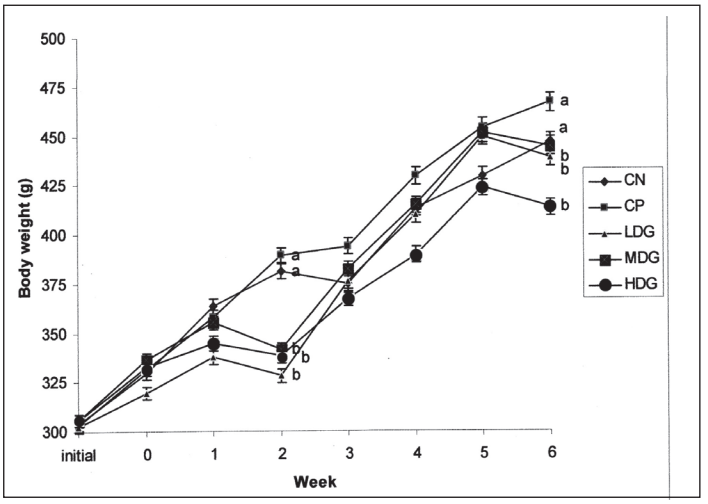

Figure 1. Mean body weight of HFD induced-obese rats supplemented with pink guava puree Superscripts with different letters are significantly different at $p<0.05$ within the same week; n=6

Note
CN : Control Negative (rat pellet + water)
CP : Control Positive (HFD + water)
LDG : Low Dosage Group (HFD + 5% pink guava puree)
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the highest dose used in the experiment did not show any toxicity effects and could be considered safe (WHO, 1992). Thus, the result suggest that the pink guava optimum dosage for puree is more than 2000 mg/kg body weight. Similar results were also reported by Norazmir,Ayub & Mamot (2009) in sub-acute studies of pink guava puree in spontaneous hypertensive rats.

Anti-obesity properties of pink guava puree

Figure 1 shows the changes in body weight of all groups during the study. Feeding a high-fat diet (HFD) containing 19% protein and 60% total fat for six weeks produced significant increases ($p<0.05$) in body weight and parametrial adipose tissue weight as compared to laboratory rat pellet (control negative, CP). HFD also induced fatty liver, with accumulation of triacylglycerol when compared to CN and CP group. In the treated group, body weight (328.11~341.29g), total cholesterol (1.35~1.60 mmol/L) and low density lipoprotein-cholesterol (0.40~0.83 mmol/L) level were reduced significantly ($p<0.05$) as compared to CN (381.28 g; 1.95 mmol/L; 0.97 mmol/L) and CP (389.45 g; 2.06 mmol/L; 1.32 mmol/L) groups. Throughout the experiment, the food and water intake of each group did not differ (data not shown).

Rats fed on a HFD experienced a significant increase in body weight gain, and pink guava puree has anti-obesity properties effects on this model. The present result clearly showed that giving pink guava puree is beneficial for the suppression of diet-induced obesity. However, this effect will be more significantly different if regular exercise is included as reported by Ono *et al.* (2006). This study indicate that the decrease in body weight might be contributed by the increased use of glucose by the tissues (Guyton & Hall, 2000). There was a marked alteration in the

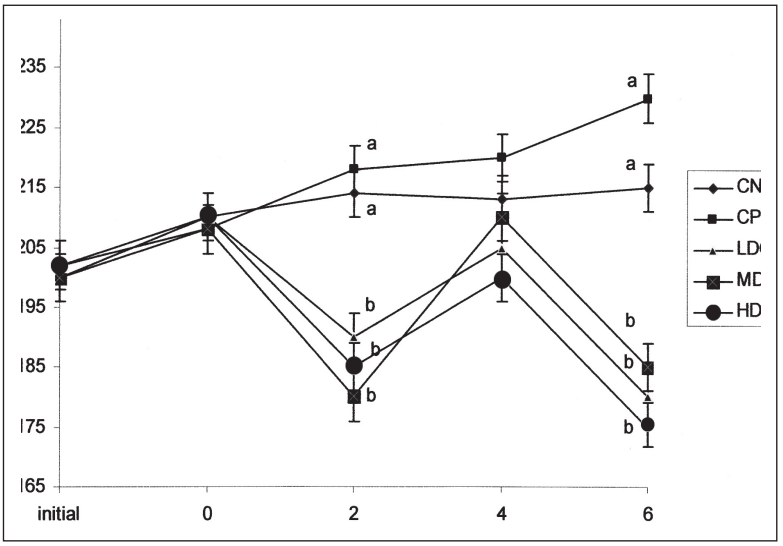

Figure 2. Systolic blood pressure value of HFD induced-obese rats supplemented with pink guava puree

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

Note
CN : Control Negative (rat pellet + water)
CP : Control Positive (HFD + water)
LDG : Low Dosage Group (HFD + 5% pink guava puree)
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HFD : High Fat Diet AIN93G

values of hematological parameters as compared to treated groups suggesting that the puree recovered the abnormal values. At the same time, liver enzymes such as AST, ALT and ALP considered responsible for cell damage are reduced by the pink guava puree and this finding suggests that the puree protects cellular damage.

Measurement of systolic blood pressure

The present study was also designed to evaluate the effects of pink guava puree on blood pressure of HFD induced-obesed rats. Figure 2 shows the blood pressure values for all groups. Systolic blood pressures for MDG, HDG and LDG were significantly lower as compared to CN and CP groups for second and sixth weeks, respectively. Blood pressure values before the treatment for CN, CP, LDG, MDG and HDG were approximately 200 ± 05 mmHg. Values for the second week showed that for all treated groups, blood pressure significantly decreased to 185 ± 05 mmHg as compared to CN and CP (216 ± 02 mmHg). The same outcome is also seen for the sixth week, whereby all treated groups demonstrated a significantly lower blood pressure value at 180 ± 05 mmHg as compared to control groups at 223 ± 07 mmHg.

Al-Awwadi, Bornet & Azay (2004) reported that polyphenols are able to prevent cardiac hypertrophy and the production of reactive oxygen-species, as well as improving vascular function. Ram *et al.* (1992) did a study on patients with essential hypertension. In their study, the guava extracts were administered before meals in a randomised and single blind fashion for twelve weeks. There was a significant net

Table 3. Blood glucose and lipid profile of HFD induced-obese rats supplemented with pink guava puree

(mmol/L)	*CN*	*CP*	*LDG*	*MDG*	*HDG*
Blood glucose	5.76 ± 1.89[a]	5.83 ± 1.17[a]	4.90 ± 0.21[b]	4.83 ± 0.58[b]	4.36 ± 0.89[b]
TC	1.95 ± 0.39[b]	2.06 ± 0.26[a]	1.60 ± 0.37[b]	1.58 ± 0.32[b]	1.35 ± 0.22[c]
TG	1.04 ± 0.40[a]	1.17 ± 1.13[a]	0.79 ± 0.18[a]	0.77 ± 0.18[a]	0.66 ± 0.16[a]
HDL-C	0.44 ± 0.13[a]	0.39 ± 0.10[a]	0.45 ± 0.10[a]	0.45 ± 0.13[a]	0.48 ± 0.07[a]
LDL-C	0.97 ± 0.39[a]	1.32 ± 0.18[a]	0.83 ± 0.22[b]	0.80 ± 0.27[b]	0.40 ± 0.08[c]

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

Note
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CP : Control Positive (HFD + water)
LDG : Low Dosage Group (HFD + 5% pink guava puree)
MDG : Medium Dosage Group (HFD + 10% pink guava puree)
HDG : High Dosage Group (HFD + 20% pink guava puree)
HFD : High Fat Diet AIN93G
TC : Total Cholesterol
TG : Triglycerides
HDL-C : High Density Lipoprotein-Cholesterol
LDL-C : Low Density Lipoprotein-Cholesterol

decrease in blood pressures values with a significant net increase in high density lipoprotein-cholesterol (HDL-C) after twelve weeks of guava fruit substitution in patient groups with essential hypertension. Previous studies have also demonstrated that pink guava has an anti-hypertensive property and was useful in reducing blood pressure (Ayub *et al.*, 2010). All the currently available evidence favours the hypothesis of the beneficial effects of pink guava puree intake in the reduction of hypertension.

Effect of pink guava puree on blood blucose and lipid profile

Blood glucose

The effects of the pink guava puree on blood glucose are shown in Table 3. Blood glucose values was significantly lowest in HDG (4.36 ± 0.89 mmol/L), followed by MDG (4.83 ± 0.58 mmol/L) and LDG (4.90 ± 0.21 mmol/L) as compared to CN (5.76 ± 1.89 mmol/L) and CP (5.83 ± 1.17 mmol/L), respectively. It showed that the puree produced a significant decrease (p<0.05) in the blood glucose levels when compared to CN and CP in the treatment study at certain dose levels.

Lipid profile

From this study, we found that daily oral administration of pink guava puree supplements showed positive results in significantly reducing total cholesterol and LDL-C levels (Table 3) after six weeks which is similar to a study by Koshy, Anila & Vijayalakshmi(2001). HDG that received higher dosage showed a 34.47% reduction in total cholesterol (TC) levels followed by the MDG (23.30%) and LDG (22.33%) respectively as compared to CP. The triglyceride (TG) levels of the treated groups showed a decrease after six weeks of treatment. The highest reduction in TG levels was in the HDG with 43.59% followed by MDG (34.19%) and LDG (32.48%). High density lipoprotein-cholesterol (HDL-C) levels showed an increase in LDG (13.33%), MDG (13.33%) and HDG (18.75%) as compared to CP. The low density lipoprotein-cholesterol (LDL-C) levels

Table 4. Kidney function test of HFD induce-obese rats supplemented with pink guava puree

	CN	*CP*	*LDG*	*MDG*	*HDG*
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]
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]
Uric acid (mmol/L)	0.38 ± 0.24[a]	0.41 ±0.19[a]	0.37 ±0.15[a]	0.34 ± 0.17[a]	0.33 ±0.06[a]

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

Note
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showed a reduction in value, for HDG (69.70%), MDG (39.40%) and LDG (37.12%) respectively as compared to CP. The higher reduction in TC levels in treated groups may be due to the increased excretion of bile acid. Pink guava is reported to have high crude fibre and mineral content, especially potassium, sodium, magnesium, phosphorus, zinc and iron (Ayub *et al.*, 2010). Ram *et al.* (1992) reported that moderate feeding of pink guava puree caused changes in dietary fatty acids and carbohydrates.

Some researchers claimed that a diet rich in vegetables and fruits can prevent atherosclerosis. However, rats fed with a HFD showed no any inhibitory effects and this may be probably due to the high concentration of cholesterol in liver and plasma because of cholesterol feeding (Zulet *et al.*, 1999). Obesity that is related to hypercholesterolemia and hypertriglyceridemia is a major risk factor for the development of cardiovascular disease. Oxidatively damaged LDLs are taken up by macrophages, which accumulate in the endothelial wall as lipid-laden foam cell in the initial phases of atherosclerotic fatty streak lesions. Therefore, a reduction in circulating TGs, TC and LDLs is primary in prevention of vascular disease. In addition, prevention of LDL oxidation by dietary antioxidants could delay the development of atherosclerosis (Koshy *et al.*, 2001). In the present study, feeding rats with HFD resulted in induced obesed rats. This model was used to study the potential of pink guava puree supplementation that contained significant amounts of antioxidants properties and useful minerals (Norazmir *et al.*, 2009; Ayub *et al.*, 2010).

Effect of pink guava puree on kidney function test

Table 4 shows significant changes ($p<0.05$) in urea concentrations in HDG (3.85 ± 0.71 mmol/L), MDG (4.35 ± 0.87 mmol/L) and LDG (4.28 ± 0.69 mmol/L) respectively, as compared to CN(7.02 ± 1.81 mmol/L). Creatinine and uric acid concentrations did not show any significant differences between treated group and CN and CP. Kidney is the second organ most frequently affected by any compounds (Marshall, 2000). Therefore, renal functions can be assessed by measuring the concentrations of creatinine and urea in plasma (Moshi *et al.*, 2001). Previous reports showed that some herbal preparations used for a long period are associated with kidney injury. 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 synthesised in the liver, primarily as a by-product of the deamination of amino acids. Creatinine, a

by-product from muscle mass, will affect its concentration in blood (Vaughn, 1999). Nzi *et al*. (2007) found that based on the biochemical analysis of renal and hepatobiliary functions, such as the levels of urea, creatinine and alkaline phosphate value, the fruit extract / juices are generally tolerated by rats. These findings are similar to this study.

Effect of pink guava puree on liver function test

Table 5 shows the activities of serum enzyme (AST, ALT, ALP, LDH), total protein, albumin, globulin, albumin:globulin ratio (AG ratio), total bilirubin, amylase and CK-Nac concentrations. There were no significant differences in all parameter between treated groups as compared to CP. There were significant differences (p<0.05) in total protein, globulin, AG ratio, ALT, LDH and CK-Nac between treated groups as compared to CN. Total protein was lower in MDG (72.67 ± 3.65 g / L), HDG (76.00 ± 2.49 g / L) and LDG (76.26 ± 3.86 g / L) respectively, as compared to CN (80.11 ± 1.98 g / L). MDG's globulin was lower (32.17 ± 1.83 g / L) followed by LDG (34.17 ± 3.43 g / L) and HDG (35.00 ± 3.41 g / L) compared to CN (39.67 ± 0.82 g / L). AG ratio was significantly higher in MDG (1.28 ± 0.07), LDG (1.22 ± 0.16) and HDG (1.19± 0.14) respectively, as compared to CN (1.03 ± 0.08). Alanine aminotransferase (ALT) was significantly lower in MDG (50.67± 22.65 U / L), LDG (55.83 ± 15.12 U / L) and HDG (57.50 ± 8.48 U / L) respectively, as compared to CN (55.83±15.12 U / L). MDG's lactate dehydrogenase (LDH) was lower in MDG (543.17 ± 295.41 U / L), LDG (766.50 ± 300.48 U / L) and HDG (928.00 ± 631.41 U / L) respectively, as compared to CN (1743.17 ± 710.49 U / L). CK-Nac were significantly lower in MDG (127.00 ± 23.55 U / L), HDG (192.50 ± 118.86 U / L) and LDG (211.00 ± 95.90 U / L) respectively, as compared to CN (487.50 ± 243.07 U / L).

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 and globulin concentrations were not affected by the pink guava puree in the treated group as 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 on *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.

Effect of pink guava puree on antioxidative enzyme activities

Table 6 shows that the specific activities of glutathione peroxidase (GPx) were significantly higher in LDG (2787.50 ± 266.36 U / L), MDG (2819.50 ± 262.04 U / L) and HDG (2897.33 ± 674.97 U / L) respectively, as compared to CN (2184.50 ± 816.59 U / L) and CP (2610.17 ± 61.63 U / L). Significant differences (p<0.05) were also seen in superoxidase dismutase (SOD) activities among all treated groups [LDG (404.67 ± 18.32 U / L), MDG (409.33 ± 55.22 U / L), HDG (418.67 ± 35.48 U / L) as compared to CN and CP]. 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 5. Liver function test of HFD induced-obese rats supplemented with pink guava puree

	CN	*CP*	*LDG*	*MDG*	*HDG*
Tpro (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]
A : G 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]
Tbil (µmol/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]
A : A ratio (U/L)	1.83 ± 0.75[a]	1.83 ± 0.41[a]	2.00± 0.63[a]	1.50 ± 0.84[a]	2.00 ± 0.11[a]
LDH (U/L)	1743.17 ±710.49[a]	430.50 ±221.64[b]	766.50 ±300.48[b]	543.17 ±295.41[b]	928.00 ±631.41[b]
CK-Nac (U/L)	487.50 ±243.07[a]	126.17 ±64.99[b]	211.00 ± 95.90[b]	127.00 ±23.55[b]	192.50 ±118.86[b]
Amylase (U/L)	1813.67 ±222.63[a]	2115.83 ±242.03[a]	2054.83 ±328.65[a]	1825.83 ±153.10[a]	1825.50 ±262.74[a]

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

Note
CN : Control Negative (rat pellet + water)
CP : Control Positive (HFD + water)
LDG : Low Dosage Group (HFD + 5% pink guava puree)
MDG : Medium Dosage Group (HFD + 10% pink guava puree)
HDG : High Dosage Group (HFD + 20% pink guava puree)
HFD : High Fat Diet AIN93G
Tpro : Total protein
Tbil : Total bilirubin
ALP : Alkaline phosphate
ALT : Alanine aminotransferase
AST : Aspartate transaminase
LDH : Lactate dehydrogenase

Table 6. Effect of pink guava puree on enzyme activities in HFD induced-obese rats

Enzyme activities	*CN*	*CP*	*LDG*	*MDG*	*HDG*
Glutathione peroxidase (U/L)	2184.50 ± 816.59^{b}	2610.17 ± 61.63^{b}	2787.50 ± 266.36^{a}	2819.50 ± 262.04^{a}	2897.33±674.97^{a}
Superoxide dismutase (U/mL)	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}
Glutathione reductase (U/L)	116.17 ± 10.76^{c}	132.50 ± 19.41^{bc}	137.33 ± 19.69^{bc}	181.00 ± 30.26^{ab}	203.00± 10.30^{a}
Total antioxidant status (mmol/L)	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
CN : Control Negative (rat pellet + water)
CP : Control Positive (HFD + water)
LDG : Low Dosage Group (HFD + 5% pink guava puree)
MDG : Medium Dosage Group (HFD + 10% pink guava puree)
HDG : High Dosage Group (HFD + 20% pink guava puree)
HFD : High Fat Diet

CONCLUSION

In conclusion, this study demonstrates that pink guava (*Psidium guajava*) puree exhibited anti-obesity properties and high enzyme activities, significantly contributing to a reduction in body weight, systolic blood pressure, TC, TG and LDL-C and increasing HDL-C levels in the role of cardiovascular disease prevention. Blood hematology, kidney and liver function test showed extensive differences in the treated group compared to the control group. Pink guava puree also showed no toxicity effects on the animals throughout the experiment.

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