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Enzyme Activities and Histology Study on High Fat Diet-induced Obese Rats by Pink Guava Puree

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Abstract: The effects of pink guava (*Psidium guajava*) puree on enzyme activities and histology on High Fat Diet (HFD)-induced obese rats were 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 HFD-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. 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. Histologically, the liver and kidney cells in LDG, MDG and HDG showed no significant differences as compared to CN and CP's liver and kidney cells. Treatment with low, medium and high doses showed improved features in HFD induced-obese rat's liver and kidney cells. In conclusion, pink guava puree due to its antioxidant role was helpful in protecting organ tissues in experimental animals and has a significant impact on specific activities of HFD induced-obese rats.

Key words: Pink guava, enzyme activities, kidney, liver, histology

INTRODUCTION

Oxidative stress is thought to contribute to the development of a wide range of diseases (Nunomura *et al*., 2006), 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 and Halliwell, 1996).

Reactive Oxygen Species (ROS) are continuously produced in biological system by the action of mitochondrial electron transport system and nicotinamide adenine dinucleotide phosphate oxidase (Cadenas *et al*., 1997). These ROS are cellular renegades and wreak havoc in biological system by tissues damage, altering biochemical compounds, corroding cell membranes and killing out rightly (Wiseman and Halliwell, 1996). To scavenge ROS, cells have several antioxidant enzymes including catalase, glutathione peroxidase, superoxide dismutase and glutathione-S-transferase.

Obesity and diabetes have reached epidemic proportions throughout the world (James, 2004). 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*., 2009b). Previous study by Asmah *et al*. (2006) showed that guava consumption could reduce oxidative stress and improve blood lipid profile.

Guava (*Psidium guajava*) is widely cultivated and Malaysia is the largest producer and exporter of pink guava puree. Guava has several carotenoids such as

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phytofluene, ß-carotene, ß-cryptoxanthin, lycopene, rubixanthin and lutein (Thaipong *et al*., 2006). In our previous study, it showed anti-hypertensive (Ayub *et al*., 2010) and lipid-lowering properties (Norazmir and Ayub, 2010a). Therefore, pink guava puree thus represents a useful way to study the effects of pink guava (*Psidium guajava*) puree on oxidative stress in targeting organ tissues in High Fat Diet (HFD)-induced obese rats.

MATERIALS AND METHODS

Pink guava puree supplement: Pink guava (*Psidium guajava*) puree from mix variety of *Beaumont Sungkai* and *Beaumont Semenyih* obtained directly from Golden Hope Food and Beverages Sdn. Bhd. Ayub *et al*. (2010) has studied the contents of the pink guava puree. The puree that was packed in a metalized (aluminum) packages was stored immediately at -70°C until the study was carried out. Once opened, the puree was repackaged into container of about 5L before make 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 -70°C until used.

Experimental procedure: Thirty male Sprague-Dawley rats each weighing between 200-280 g obtained from UKM animal house were kept one per metabolic cages in a temperature-controlled room at 25±2°C with a 12:12 h 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 h) 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 (Norazmir and Ayub, 2010b). The organs 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.

Analytical procedures: HFD induced-obese rats were fasted overnight (12-14 h) 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. The plasma samples were kept frozen at -70°C until used. Serum was obtained by collecting blood in non-EDTA tube. The serum was used for determine kidney and liver function test. Plasma and serum samples were kept at -70°C. 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.

Histology of kidney and liver: Histological examination was based on an earlier protocol (Humason, 1979). Slices of the liver lobe and kidney were fixed in Bouin's solution for 24 h. All samples were then dehydrated in graded ethanol series, cleared in toluene and embedded in paraffin; 5-6 µm sections were routinely stained with Trichrome Stains (Masson) (Sigma-Aldrich) and were assessed under light microscope (Nikon Eclipse E400).

Statistical analysis: Data was analyzed using SAS system. The significant differences between the control and treated groups were analyzed using Duncan's Multiple Range Test. All mean values were expressed as group means ± Standard Error of Mean (SEM). The minimal level of significance accepted was $p<0.05$.

RESULTS AND DISCUSSION

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 1, mean body weights almost 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.

Organ's relative weight such as liver, heart, kidney, lung, spleen and testes (Table 1) 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

Table 1: Effects of pink guava puree on body and organ weights in High Fat Diet (HFD) induced-obese rats

Body weight (g)	CNG	CPG	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	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. CNG = Control Negative Group (pellet + water); CPG = Control Positive Group (HFD + water); LDG = Low Dose Group (HFD + 5% pink guava puree); MDG = Medium Dose Group (HFD + 10% pink guava puree); HDG = High Dose Group (HFD + 20% 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 and Ayub (2010) also reported similar results in sub-acute studies of pink guava puree in spontaneous hypertensive rats.

Blood haematology: Blood hematology showed significant differences in HDG's red blood cell, hemoglobin and hematocrit amount (Table 2) compared to CN group. HDG's red blood cell count (9.20±0.53 $x10^{12}$/l) was significantly different compared to CN group (10.78±1.38 $x10^{12}$/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 the *Psidium guava* extract has no adverse effect on the HFD-induced obese rats.

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 3. 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 increases in the activities of erythrocyte CuZn-SOD and GPx after 10 weeks on the diet. It attributed the increases 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. and Vincent et al. 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 an increase in the levels of glutathione, which is similar to this study.

Kidney function tests: Kidney function tests 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 4. Creatinine and uric acid concentrations did not show any significant differences between supplemented pink guava-treated rats

Table 2: Blood hematology of High Fat Diet (HFD) induce-obese rats supplemented with pink guava puree

	CNG	CPG	LDG	MDG	HDG
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. 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 (%).
CNG = Control Negative Group (pellet + water); CPG = Control Positive Group (HFD + water); LDG = Low Dose Group (HFD + 5% pink guava puree); MDG = Medium Dose Group (HFD + 10% pink guava puree); HDG = High Dose Group (HFD + 20% pink guava puree)

Table 3: Effect of pink guava puree on enzyme activities in High Fat Diet (HFD) induce-obese rats

	CNG	CPG	LDG	MDG	HDG
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. GPx : Glutathione Peroxidase (U/L); SOD: Superoxide Dismutase (U/L); GR : Glutathione Reductase (U/L); TAS : Total Antioxidant Status (mmol/L).
CNG = Control Negative Group (pellet + water); CPG = Control Positive Group (HFD + water); LDG = Low Dose Group (HFD + 5% pink guava puree); MDG = Medium Dose Group (HFD + 10% pink guava puree); HDG = High Dose Group (HFD + 20% pink guava puree)

Table 4: Kidney function test of High Fat Diet (HFD) induce-obese rats supplemented with pink guava puree

	CNG	CPG	LDG	MDG	HDG
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}	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.
CNG = Control Negative Group (pellet + water); CPG = Control Positive Group (HFD + water); LDG = Low Dose Group (HFD + 5% pink guava puree); MDG = Medium Dose Group (HFD + 10% pink guava puree); HDG = High Dose Group (HFD + 20% pink guava puree)

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.

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 and 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.

Liver function tests: 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 5. 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 and Ayub, 2010b). Liver function test is crucial because liver

Table 5: Liver function test of High Fat Diet (HFD) induce-obese rats supplemented with pink guava puree

	CNG	CPG	LDG	MDG	HDG
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 (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±16.18[a]

Means with the same letter in same row are not significantly different ($p<0.05$); n=6. ALP : Alkaline Phosphate; ALT : Alanine Aminotransferase; AST : Aspartate Aminotransferase.

CNG = Control Negative Group (pellet + water); CPG = Control Positive Group (HFD + water); LDG = Low Dose Group (HFD + 5% pink guava puree); MDG = Medium Dose Group (HFD + 10% pink guava puree); HDG = High Dose Group (HFD + 20% pink guava puree)

Table 6: Urine profile of High Fat Diet (HFD) induce-obese rats supplemented with pink guava puree

Urine profile components	CNG	CPG	LDG	MDG	HDG
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	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. -ve : negative.

CNG = Control Negative Group (pellet + water); CPG = Control Positive Group (HFD + water); LDG = Low Dose Group (HFD + 5% pink guava puree); MDG = Medium Dose Group (HFD + 10% pink guava puree); HDG = High Dose Group (HFD + 20% pink Guava puree)

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 *et al*., 2009b). 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).

Urine profile: Table 6 showed the urine profile between dosage groups on the last day of experiment. No glucose and blood were 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 and 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*. (2009a) study using pink guava puree on spontaneous hypertensive rats.

Histology analysis: Liver and kidney are important organs of metabolism, detoxification, storage and excretion of xenobiotics and their metabolites, and are especially vulnerable to damage (Brzoska *et al*., 2003). Photomicrograph of rat kidney in control group and treated group were closely observed (Fig. 1). Normal structure of the kidney was observed in the kidney of control rats. Renal corpuscles, proximal and distal convoluted tubules can be seen in the sections. No significant changes were observed in the kidney histology of rats of the treated groups. Figure 2 showed photomicrograph of rat liver in control group and treated groups. Microscopic observations showed a normal liver histomorphology in control rats. In treated rats, liver showed normal structure of liver tissue composed of hexagonadal or pentagonadal lobules with central veins and peripheral hepatic triads or tetrads embedded in connective tissue. Hepatocytes are arranged in trabecules running radiantly from the central vein and

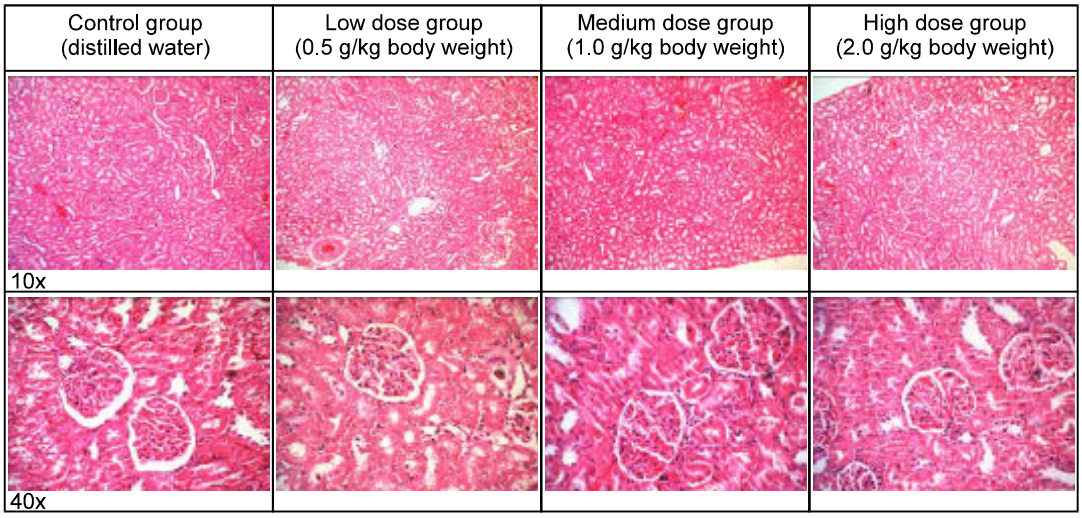

Fig. 1: Photomicrograph of kidney from the control group and treated groups (10x and 40x magnifications). No significant damage was detected in any treatment group. Trichrome stain (a) x 10 (b) x 40

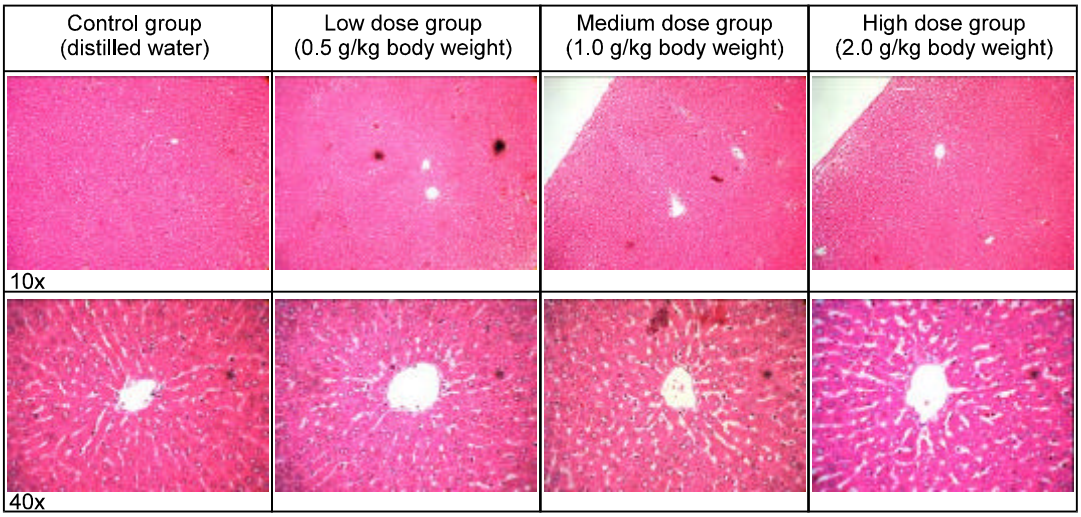

Fig. 2: Photomicrograph of liver from the control group and treated groups (10x and 40x magnifications). No significant damage was detected in any treatment group. Trichrome stain (a) x 10 (b) x 40

are separated by sinusoids containing Kupffer cells. They are regular and contain a large spheroidal nucleus with a distinctly marked nucleolus and peripheral chromatin distribution. Histopathological examinations of the liver revealed no pathological abnormality of the low, medium and high dose treatment groups as compared with the control groups.

Conclusion: Pink guava puree supplements seem to be beneficial for increased antioxidant enzyme activities with significant result to reduce body weight. Blood haematology, kidney and liver function test showed extensively differences in treated groups as compared to control groups. Histology analysis of the liver and kidney revealed no pathological abnormality as compared to with the control groups. These results suggest that the guava puree did not cause toxicities in rats. In conclusion, pink guava puree due to its antioxidant role was helpful in protecting tissues in experimental animals from oxidative stress and has a significant impact on health status of HFD induced-obese rats.

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