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Effect of selected tropical fruits on health-promoting properties in rats

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Abstract
Guava, papaya and soursop are among several tropical fruits that contain antioxidants. The study was conducted to investigate the health-promoting effect of these fruits on *Sprague-Dawley* rats. The parameters determined were total antioxidant status, alanin aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin, globulin, urea, creatinine, total cholesterol, triglyceride, low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C). TAS level in supplemented (guava, papaya and soursop) group were significantly higher (p <0.05) compared to the control group. Supplementation of guava, papaya and soursop puree did not affect the ALT and AST levels. Significant difference (p <0.05) was observed between control and supplemented groups for total protein, albumin and globulin levels. The total cholesterol level was significantly reduced (p <0.05) in guava and soursop at medium and high dosage compared to control group. Rats supplemented with papaya puree at all dosage exhibited significantly higher (p <0.05) level of HDL-C compared to control group. Supplementation with these fruits will increase the antioxidant and HDL-C level in blood serum of rats, which will improve health to a better status.

Keywords: health-promoting properties, tropical fruits, blood biochemistry, lipid profile, *Sprague-Dawley*

Introduction

Malaysia is known for its large diversity of tropical fruits. Fruit intake in diet is important because they are rich in vitamins, provitamins and phenolic compounds. These compounds can function as antioxidant agents. Other than vitamin C, the main contributor to antioxidant in fruits comes from varieties of phytochemical compound. Fruits and vegetables contain high antioxidant such as phenolic groups, carotenoids and tocopherol (Leong and Shui 2002).

Epidemiological studies showed that the best protection from fruits is its functional against cancer, stroke and coronary heart disease. This is also closely related to the antioxidants in food. Although majority of studies stated the importance of vitamin C and vitamin E, the presence of antioxidant and phenolic compound also plays an important role. Fruit juice is a good

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example of food product that is suitable for digestion and health protection because of its high absorption of bioactive components. Contents of fruits are important in healing diseases and fruit extracts or juices had shown a significant antioxidant activity (Jimenez-Escrig et al. 2001).

Determination of antioxidant properties in tropical fruits has been done using different analysis on varieties of fruits (Jimenez-Escrig et al. 2001; Someya et al. 2001; Leong and Shui 2002). These experiments showed that tropical fruits were proven to have high antioxidant content, as studied by Lim et al. (2007) on tropical fruits such as banana, starfruit, *langsat*, papaya, water lily and guava.

Preliminary study has been done on antioxidant activities of several tropical fruits such as guava, papaya, starfruit, soursop, mango, water lily, *pulasan, rambutan* and sapodilla. Five fruits that contain high antioxidant are soursop, guava, starfruit, red mango and papaya in descending order. Only three fruits were selected in this study, namely soursop, guava and papaya. The selection was based on the antioxidant activity, availability in the market and functional properties. Therefore, the objective of this study was to determine the effect of serum supplemented with guava, papaya and soursop.

Materials and methods

The tropical fruits selected in this study were guava (*Psidium guajava* L.), papaya (*Carica papaya* L.) and soursop (*Anona muricata* L.). These fruits were deseeded and peeled except for guava (unpeeled). The fresh puree was prepared by blending the fruit pulp using Warring blender for further analysis.

The puree was then diluted with distilled water according to the dosage for safety study. Each group contained different percentage of puree, where low dose (LD) group contained 5%, medium dose (MD) 10% while high dose (HD) contained 20% according to OECD Guidelines (407).

All procedures concerning the use of animals were approved by the University Kebangsaan Malaysia Animal Ethic Committee (UKMAEC). These animals were housed in a controlled environment, with temperature of 24 ± 2 °C and a relative humidity of 30 – 70%. The rooms were illuminated with 12 h artificial fluorescent light and 12 h darkness per day. The animals were provided with a standard pelleted laboratory animal diet and distilled water *ad libitum*. The animals were allowed to acclimatise for 7 days before starting the treatment.

The sub-acute or 28 days repeated dose study was performed based on Ryu et al. (2004). Five male *Sprague-Dawley* rats weighing 200 – 250 g per group were given distilled water (control dose, CD), 5% low dose of guava (GL), papaya (PL) and soursop (SL); 10% medium dose of guava (GM), papaya (PM) and soursop (SM); and 20% high dose of guava (GH), papaya (PH) and soursop (SH) of puree via drinking bottles as they can access *ad libitum*. Each rat would get 100 ml of puree per day. Any remaining puree left would be measured. The physical conditions of each animal were monitored daily and the body weight was measured weekly. On day 29, all rats were sacrificed and blood samples were collected from posterior *vena cava*.

For biochemistry analyses, animals were fasted for approximately 12 h and blood samples were withdrawn from posterior vena cava. Samples of blood were withdrawn under light ether anesthesia (Ryu et al. 2004).

For the evaluation of biochemical parameters, one aliquot of blood per animal was placed in a 5 ml Z-serum tube (Bacton Dickinson, BD Vaccutainer) and centrifuged at 15,000 rpm for 20 min. Serum aliquots were subjected to evaluation of alanine amino transferase (ALT), aspartate amino transferase (AST), lactate dehydrogenase (LDH), γ-glutamyl transferase (GGT) and alkaline phosphatase (ALP) activities, total protein, albumin, globulin, albumin/

globulin (A/G) ratio, urea, creatinine, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglyceride. All parameters were measured using Blood Clinical Analyzer (Vitalab Selectra E). The reagents for the tests were obtained from Randox (Randox Laboratories Ltd, Antrim, United Kingdom).

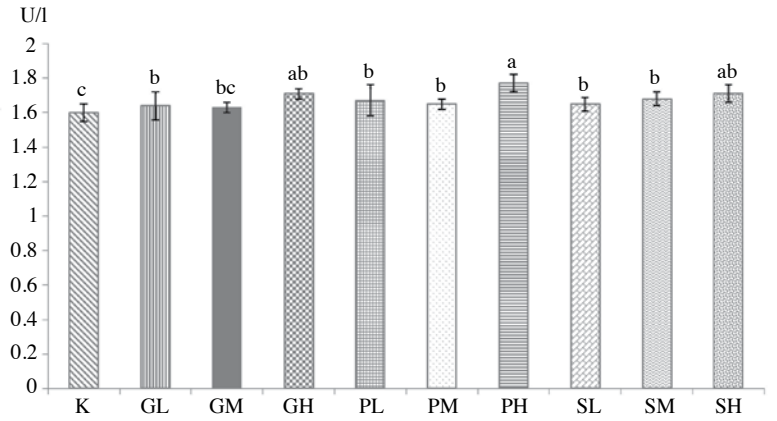
Statistical analysis

All data were subjected to one-way analysis of varians (ANOVA) and Duncan by using the Statistical Analysis System (SAS v.11).

Results and discussion

Level of total antioxidant status (TAS) in rats supplemented with puree was significantly higher (p <0.05) compared to control group. All high dosage of purees (GH 1.71 ± 0.03 U/l, PH 1.77 ± 0.05 U/l and SH 1.71 ± 0.05 U/l) showed a significantly higher (p <0.05) of TAS compared to control group (1.60 ± 0.05 U/l). *Figure 1* shows that supplementation with guava, papaya and soursop puree can increase antioxidant level in blood. According to Lim et al. (2007), guava and papaya are among the tropical fruits that contain high total phenolic content (TPC). This study also showed that these fruits had a low DPPH IC_{50} value which meant a very potent radical scavenger. This might be the reason for the increase in TAS value in all rats supplemented with high dose of guava, papaya and soursop. Research done by Isabelle et al. (2010) also found a high antioxidant activity in guava and soursop in hydroxyl radical capacity antioxidant (H-ORAC) assay and TPC test. While in papaya, the antioxidant activity was contributed from content of β-cryptoxanthin, lycopene and β-carotene (Isabelle et al. 2010).

Figure 2 shows the results of enzymatic activity of liver in serum. Serum activities of ALT and AST are the most important test for diagnosing any liver or hepatic disease. The ALT for guava (GL 64.00 ± 7.00 U/l, GM 70.33 ± 2.89 U/l, GH 66.67 ± 3.51 U/l), papaya (PL 62.00 ± 8.22 U/l, PM 62.67 ± 9.02 U/l, PH 56.80 ± 8.67 U/l) and soursop (SL 58.60 ± 9.45 U/l, SM 56.20 ± 9.20 U/l, SH 53.60 ± 12.10 U/l) puree at all dosage were not significantly different compared to control group which indicated no significant effect to liver function. The increase in serum ALT level is often associated with several medical problems such as viral hepatitis, heart failure, liver damage and myopathy.



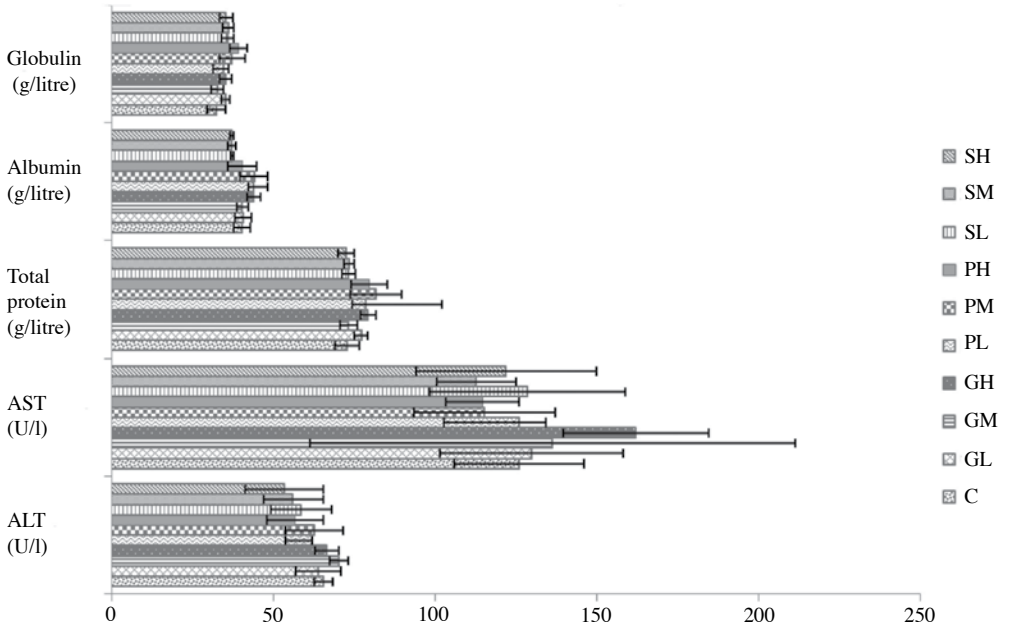
C = control group, GL = guava low dose, GM = guava medium dose, GH = guava high dose, PL = papaya low dose, PM = papaya medium dose, PH = papaya high dose, SL = soursop low dose, SM = soursop medium dose, SH = soursop high dose

Figure 1. Total antioxidant status in serum

The AST enzyme is released into the blood stream when any tissue or organ such as heart or liver is damaged or infected. The total AST in blood is indirectly proportional to the organ damaged (Patel et al. 2008). No significant difference in AST was observed between rat groups fed with different levels of guava, papaya and soursop puree. Different dosage of puree did not show any significant effect on AST for treated rats (*Figure 2*).

For total protein, albumin and globulin analyses, decreased level of these parameters (Patel et al. 2008) in blood indicates cirrhosis or liver malfunction. Levels of total protein in the serum given purees of different fruits and dosage were not significantly different compared to control except for group fed with all doses of papaya and high dose of guava (*Figure 2*). A high protein level shows that there is liver and kidney malfunction when proteins are not completely digested or absorbed. A lower protein level is usually related to dehydration or myeloma multiple (Petterino and Argentino-Storino 2006).

The level of albumin was not significantly different for all treated groups when compared to control group (*Figure 2*). Groups given soursop puree were significantly lower ($p < 0.05$) in albumin compared to high dose of guava and also medium and high dose of papaya puree. The increment of albumin level in blood is known as hiperalbuminemia. The interpretation of this condition is related to a chronic dehydration or chronic liver disease (Anon. 2012). The globumin level for groups with PM, PH and SM were significantly higher ($p < 0.05$) than control group. The globulin level was not significantly different between all the three fruits (*Figure 2*).



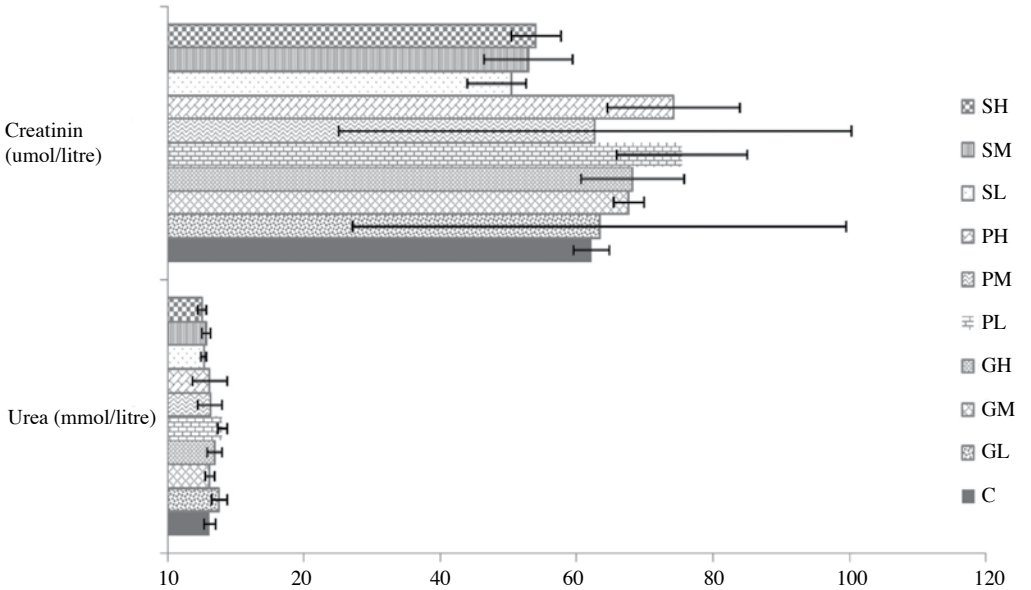
C = control group, GL = guava low dose, GM = guava medium dose, GH = guava high dose, PL = papaya low dose, PM = papaya medium dose, PH = papaya high dose, SL = soursop low dose, SM = soursop medium dose, SH = soursop high dose, ALT = alanine aminotransferase, AST = aspartate aminotransferase

Figure 2. Enzymatic activity of liver in serum of rats

The significant difference (p <0.05) between control group and puree group of total protein, albumin and globulin level was not associated with any liver failure or malfunction. The levels of total protein, albumin and globulin were within the normal range 61 – 87, 30 – 44 and 30 – 36 g/litre respectively (Petterino and Argentino-Storino 2010). The levels of ALT and AST in purees group that were not significantly different to control group also confirmed that administration of guava, papaya and soursop puree did not result in any liver failure. The results suggest that consumption of guava, papaya and soursop puree did not affect or cause any liver damage to the rats.

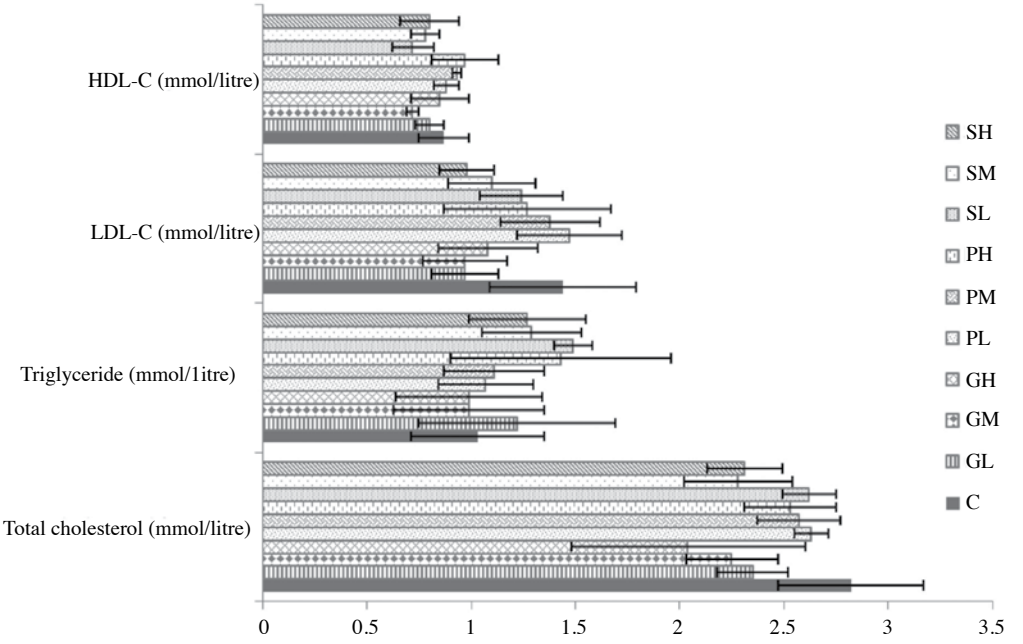
Urea and creatinin are parameters used to determine kidney failure. A high level of urea and creatinin in blood serum indicates the occurrence of severe kidney damage. The levels of urea in rats given guava, papaya and soursop puree were not significantly different compared to control group except for PL group (*Figure 3*). Results between purees showed that urea level in rats given PL was significantly higher than PM, PH, GM and all dose of soursop puree. There was no significant difference between all dosage of guava and soursop puree. The guava, papaya and soursop puree in all dosage did not differ significantly in creatinin level. The main reason for the increase in the urea level in blood is due to the kidney failure. High increment of urea level of more than 31.7 mmol/litre referred to the degree of damage from medium to high kidney damage. The increase of urea level at normal creatinin level did not relate to any kidney failure or damage (Petterino and Argentino-Storino 2010).

Figure 4 shows a lipid profile in blood of rats given guava, papaya and soursop puree. The total cholesterol level was significantly reduced (p <0.05) in guava and soursop in both medium and high dose compared to control group. Reduction in total cholesterol was also noted in each fruit puree as the dosage increased. The results did not show any significance



C = control group, GL= guava low dose, GM = guava medium dose, GH = guava high dose, PL = papaya low dose, PM = papaya medium dose, PH = papaya high dose, SL = soursop low dose, SM = soursop medium dose, SH = soursop high dose

Figure 3. Urea and creatinin levels in serum of rats



C = control group, GL = guava low dose, GM = guava medium dose, GH = guava high dose, PL = papaya low dose, PM = papaya medium dose, PH = papaya high dose, SL = soursop low dose, SM = soursop medium dose, SH = soursop high dose

Figure 4 Lipid profile of serum rats

of total cholesterol between the three fruits. Consumption of these purees did not give any significant effect on triglyceride and LDL-C level as compared to control group. Rats given papaya puree in all dosage had significantly higher (p <0.05) level of HDL-C compared to control group. Studies done by Argawal and Rao (1998) and Fuhram et al. (1997) using papaya also showed the same pattern. According to their research, combination of carotenoids in papaya such as lycopene, β-cryptoxantin and lutein plays a main role in reducing LDL-C level in blood.

Conclusion

Consumption of guava, papaya and soursop puree increased the TAS level in blood. At different dosage, all purees did not give any negative effect to kidney and liver of rats. These purees have the ability in reducing total cholesterol level as the dosage increase when compared to control group. High dose of papaya puree can increase the level of HDL-C compared to control.

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Abstrak

Jambu, betik dan durian belanda merupakan beberapa buah tempatan yang mengandungi antioksidan. Kajian ini dijalankan untuk menentukan kesan buah bagi meningkatkan kesihatan tikus *Sprague-Dawley*. Parameter yang ditentukan ialah jumlah status antioksida (TAS), alanin aminotransferase (ALT), aspartate aminotransferase (AST), jumlah protein, albumin, globulin, urea, kreatinin, jumlah kolesterol, trigliserida, lipoprotein-kolesterol berketumpatan rendah (LDL-C) dan lipoprotein-kolesterol berketumpatan tinggi (HDL-C). Nilai TAS dalam kumpulan suplemen (jambu, betik dan durian belanda) lebih signifikan tinggi (p <0.05) berbanding dengan kumpulan kawalan. Suplemen puri jambu, betik dan durian belanda tidak memberi kesan pada nilai ALT dan AST. Perbezaan signifikan (p <0.05) diperhatikan antara kumpulan kawalan dengan suplemen untuk nilai jumlah protein, albumin dan globulin. Nilai jumlah kolestrol) menurun secara signifikan (p <0.05) dalam jambu dan durian belanda pada dos sederhana dan tinggi berbanding dengan kumpulan kawalan. Tikus yang diberi suplemen puri betik pada kesemua dos menunjukkan peningkatan signifikan (p <0.05) pada nilai HDL-C berbanding dengan kumpulan kawalan. Suplemen buah-buahan ini meningkatkan kandungan antioksidan dan HDL-C di dalam serum darah tikus yang mampu meningkatkan status kesihatan yang lebih baik.