International Food Research Journal 22(4): 1318-1326 (2015)
Journal homepage: *http://www.ifrj.upm.edu.my*

Protective effects of pulp and kernel oils from *Canarium odontophyllum* fruit in normal and hypercholesterolemic rabbits

[1]Faridah Hanim, S., [2,3,4,*]Azrina, A., [2]Khoo, H. E. and [2,3,4]Amin, I.

[1]*Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, UPM Serdang, 43400 Selangor, Malaysia*
[2]*Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, UPM Serdang, 43400 Selangor, Malaysia*
[3]*Laboratory of Halal Science Research, Halal Products Research Institute, Universiti Putra Malaysia, UPM Serdang, 43400 Selangor, Malaysia*
[4]*Research Centre of Excellence, Nutrition and Non-Communicable Disease, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

Article history

Received: 14 June 2014
Received in revised form: 2 January 2015
Accepted: 12 January 2015

Keywords

Canarium odontophyllum
Hepatotoxic
Extraction
Liver function test
Oil
Protective effect

Abstract

This study aimed to determine the protective effects of CO pulp and kernel oils supplementation to normocholesterolemic and hypercholesterolemic rabbits. Rabbits from the treatment groups were supplemented with CO pulp and kernel oils for four weeks. Bloods were drawn from all experimental groups at baseline and fourth week to determine protective effects of CO oils supplementation on plasma total antioxidant status (TAS) and catalase (CAT) activity. Liver function tests (ALT, AST, and GGT activities) were also determined for all the groups. The results showed that CO oil supplementation increased plasma TAS in both normal and hypercholesterolemic groups. Plasma CAT activities in the hypercholesterolemic groups supplemented with CO oils were significantly reduced but not for the normocholesterolemic groups. Significant reduction of plasma AST was observed for the hypercholesterolemic rabbits given CO pulp and kernel oils compared with the hypercholesterolemic control rabbits, but not for plasma ALT and GGT. In the normocholesterolemic rabbits, CO pulp oil had caused a significant elevation of plasma ALT, AST, and GGT levels as compared to the negative control rabbits. Therefore, CO pulp and kernel oils are somehow not hepatotoxic, and the oils are potent functional foods.

Introduction

In nature, some fruits contain high fat content. High fat fruit is generally known to contain more than 20% of fat, and the exact amount of fat in high fat fruit has not been defined. Oil palm is one of the commonly known high fat fruit. The fat extracted from palm fruits and kernels has been used for several purposes, such as cooking oil, edible spread, and making chocolate. Since last few decades, the oil extracted from oil palm has high trade value (Carter *et al*., 2007). Besides oil palm, there are some of the high fat fruits that commercially important.

Canarium odontophyllum (CO) fruit, also known as dabai, is a high fat fruit. About 25% of fat has been extracted from the CO pulp (Chew *et al*., 2011). The extracted edible oil of CO fruit has a similar percentage of saturated fat (44%) as in palm oil (48%) while its kernel also contain fat with a higher percentage of saturated fat (61%) (Azlan *et al*., 2010). Low intake of saturated fat from plant is less likely be associated with increased risk of heart diseases compared to animal fat as the plant fat is rich in phytochemicals (Hu, 2003). Polyphenol-rich olive oil has been associated with reduced risk of heart disease (Covas *et al.*, 2006). Recently, Shakirin *et al.* (2012a) reported that pulp and kernel oils of CO have high total phenolic contents (202.7 and 39.4 mg GAE/kg oil, respectively). In addition to the TPC, they also found that CO pulp oil has a protective effect against cardiovascular risk in hypercholesterolemic rabbits. Supplementation of the CO pulp oil (2%) in normocholesterolemic rabbits for four weeks in the diet was shown to significantly improve the plasma lipids of the animal model by reducing TG, LDL-c, and MDA levels compared to normal diet rabbits. The plasma HDL-c and total antioxidant status levels were also increased significantly (Shakirin *et al*., 2012b)

Toxicity of oil extracted from fruit pulp rarely occurs as it is commonly consumed in our daily diet. By comparison, the edible fat extracted from fruit seed is more toxic than the fruit as a whole because seeds tend to contain higher amount of terpenoids than the fruit

*Corresponding author.
Email: *azrinaaz@upm.edu.my*

(Knio *et al*., 2008). Most seeds and kernels of fruits are toxic as it contains compounds such as cyanide in Indian almond nut *(Prunus amygdalus)* (Agunbiade *et al*., 2006), as well as saponin and toxic glycosides, in African walnut *(Tetracarpidium conophorum)* (Malu *et al.*, 2010). Another example is *Pangium edule* nut that commonly consumed in Papua New Guinea was reported as toxic and require removal of the toxic compound such as cyanogenic glycoside before its consumption (Lim, 2013). Famous high fat fruit such as oil palm *(Elaeis guineensis)* is broadly utilized as a source of oil and fat both from its fatty flesh and kernel. The oil palm fruit contains a high percentage of edible oil (22%) that assumed to be not toxic. Pokorný (1991) concluded that most food may have low toxicity though the foods are believed to be non-toxic.

The extracted oils of CO have excellent antioxidant properties and offer some protection against oxidative stress. CO oil is also edible and should be not toxic to the human body. Hepatotoxicity and protective activities of CO oils have not been reported using in vivo model. Therefore, this study was aimed to determine protective effect and hepatotoxicity of CO pulp and kernel oils in normocholesterolemic and hypercholesterolemic rabbit model.

Materials and Methods

Preparation of fruit

Fresh fruit of CO (60.0 kg) were obtained from Agriculture Research Centre, Sarawak, Malaysia. The fruits were packed in cool-box container and delivered to Universiti Putra Malaysia by flight. Different parts of the fruit were prepared: (1) full-fat pulp, (2) defatted pulp, (3) pulp oil, and (4) kernel oil. Briefly, the preparation started with separating pulp and kernel parts. Fruit pits were crushed mechanically to obtain a white-greenish kernel and the pulp was pooled and mashed. Both pulp and kernel samples were freeze-dried using a 35XL freeze dryer (Virtis Co. Inc. NY, USA) at Forestry Research Institute of Malaysia (FRIM). The dried pulp and kernel of CO were grinded into powders and stored in an air-tight container to prevent oxidation before analyzes.

Extraction of crude oil from the pulp and kernel of CO

CO pulp or kernel powder (100.0 g) was weighed and extracted with 500 ml of chloroform:methanol (2:1, v/v). In order to get enough oil for the animal study, ~4 kg of each CO pulp and kernel powders were pooled to yield the oils for experiment. The mixtures were soaked overnight in the solvent mixture at room temperature in a container covered with aluminium foil and filtered using Whatman No 1 filter paper (Whatman, Maidstone, UK). The organic solvents were completely evaporated using a rotary evaporator (Buchi, Berlin, Germany) at 40˚C. The residue was re-soaked with fresh solvent again twice to ensure complete extraction of the oil.

Experimental design

Male New Zealand white rabbits weighing 1.5-1.7 kg at age of 8-10 weeks were purchased from East Asia Company, Malaysia. A total of 49 male rabbits was placed in individual and stainless steel cages at animal house of Faculty of Medicine and Health Sciences, UPM. The animals were placed in a well-ventilated room with 12 h light-dark cycle, and they were given free access to tap water. The animals were acclimatized for two weeks to standardize their body weight. After acclimatization, the animals were randomly distributed into seven groups of normal and hypercholesterolemic groups. Three normal rabbit groups (NC, NP, and NK) were received normal basal diet while four hypercholesterolemic groups (HC, HP, HK, and HS) received hypercholesterolemic diet (basal diet containing 0.5% of cholesterol).

Both normal and hypercholesterolemic rabbits of the treatment groups received CO pulp and kernel oils (2% of fat as follows; NP received normal basal diet + pulp oil; NK received normal basal diet + kernel oil; HP received hypercholesterolemic diet + pulp oil; and HK received hypercholesterolemic diet + kernel oil); and HS received hypercholesterolemic diet + 10 mg/kg/day simvastatin. Control groups comprised of a normal group (NC; normal chow) and hypercholesterolemic control (HC). All experimental protocols involving animals were approved by the Animal Care and Use Committee of the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (UPM), Serdang, Selangor. (Approval no.: UPM/FPSK/PADS/BR-UUH/00238)

Preparation of animal diets

Commercialized rabbit chow and food ingredients (soybean, corn, palm kernel cake, tapioca starch, molasses, corn oil, mineral mix, and vitamin mix) were purchased from East Asia Company, Malaysia. DL-methionine, calcium carbonate, and calcium hydrogen phosphate were purchased from Merck (Darmstadt, Germany).

In the present study, two types of diets were used: commercialized rabbit chow and experimental diet (Table 1). The commercialized rabbit chow was given during the two weeks of acclimatization and the experimental diet was given during eight weeks of the treatment period. The experimental

Table 1. Formulation of experimental diet (g)

Ingredients	Experimental diet (g)						
	NC	NP	NK	HC	HP	HK	HS
Soybean meal	150	150	150	150	150	150	150
Corn	300	300	300	300	300	300	300
Palm kernel oil	360	360	360	355	355	355	355
Starch	100	100	100	100	100	100	100
Molasses	20	20	20	20	20	20	20
Corn oil	20			20			20
Vitamin mixture[2]	3	3	3	3	3	3	3
Mineral mixture[3]	35	35	35	35	35	35	35
DL-methionine	2	2	2	2	2	2	2
$CaCO_3$	5	5	5	5	5	5	5
$CaHPO_4$	5	5	5	5	5	5	5
Cholesterol				5	5	5	5
Pulp oil		20			20		
Kernel oil			20			20	
Total	1000	1000	1000	1000	1000	1000	1000

diets were prepared based on a normal basal diet. The normal basal diet contain soybean (15%), corn (30%), starch (10%), molasses (2%), corn oil (2%), vitamin mixture (0.3%), mineral mixture (3.5%), DL-methionine (0.2%), calcium carbonate (0.5%), and calcium hydrogen phosphate (0.5 %). The diet consisted vitamin mixture of vitamin A (50000 i.u.), vitamin D3 (8000 i.u.), and vitamin E (8 mg); while mineral mixture consisted manganese (320 mg), zinc (200 mg), magnesium (1400 mg), ferrous (300 mg), copper (50 mg), cobalt (10 mg), iodate (20 mg), phosphorus (10000 mg), salt (5500 mg), and calcium (1300 mg).

The basal diet for the NC group was prepared as follows; soybean, corn, and palm kernel cake were grinded using an electric grinder, weighed, and mixed manually with mineral and vitamin mixtures (mineral mixture, vitamin mixture, $CaCO_3$, $CaHPO_4$, and DL-methionine). Starch (10%) was added to the mixture together with oil and molasses, mixed well and placed carefully on a dish covered with aluminium foil. The dough was cut into small pieces and dried in an oven (Memmert GmbH & Co. KG, Schwabach, Germany) at 45-50°C overnight. The prepared pieces of the basal diet were stored in air-tight containers at room temperature. For hypercholesterolemic diet, cholesterol (0.5%) was mixed as the additional ingredient.

Similarly, the experimental diets were prepared using the same method as described for the NC diet with some modifications and the addition of other food ingredients in a particular diet. HS group was given hypercholesterolemic diet with simvastatin (10 mg/kg per day) orally by force-feeding. The simvastatin was prepared by dissolving simvastatin with distilled water. For the NP, HP, NK, and HK diets, 2% of pulp and kernel oils extracted from the pulp and kernel of CO were added, respectively. The oils given in each group replaced part of the total fat required for rabbits. The amount of fat supplementation was determined based on the method described by Zhao *et al*. (2008).

The cholesterol diets were prepared by dissolving the cholesterol in chloroform. Briefly, 0.6 g of cholesterol was suspended in 2 ml chloroform. The mixture was stirred with the aid of a magnetic stirrer at room temperature. Later, the dissolved cholesterol (representing 0.5% of the cholesterol in a daily diet) was sprayed on 20 g of normal basal diet for each animal's diet. The pellets were dried in oven at 40˚C overnight to allow the evaporation of chloroform. The diets were freshly prepared every three days. Numerous studies have used 0.25-2% of cholesterol level to induce hypercholesterolemia in animal models (Gonzalez-Santi *et al.*, 2006; Shimizu *et al*., 2009; Prasad, 2010). However, 0.5% of cholesterol level was chosen in the present study as this was enough to induce mild atherosclerosis plaque.

Food intake and body weight

Each day, 20 g of the cholesterol containing pellets were given early in the morning and were ensured to be finished by the rabbits before they were given the remaining 100 g of the experimental diet. Food intake of each animal was measured daily by weighing the food residue. The amount of food given was 120 g daily, and the intake of each animal was calculated by subtracting the amount of food residue from 120 g of the food given. The animals' body weights were recorded at baseline and week four of the study. A 4-week period was selected based on a study reported by Basu *et al.* (2007), where the protective effects of hypercholesterolemic rabbits were not significantly differed between 4-week and 8-week of the study.

Blood collection

Blood was drawn from the marginal ear of rabbits at week 0 (baseline) and week four for all groups. Rabbits were fasted for at least 12 hours before the blood sampling. The volume of blood taken was approximately 9.0 ml. The blood collected in tubes containing lithium heparin were used for determination of plasma catalase activity and liver function enzymes. The collected blood was centrifuged at 3000 rpm for 10 min at 25˚C using a refrigerated centrifuge (Universal 32, Hettich Zentrifugen, Germany) to separate the plasma.

Total antioxidant status

Total antioxidant status (TAS) was determined

in heparinized plasma of the experimental rabbits using Randox kit and measured by clinical chemistry analyzer (Vitalab Selectra E, Germany). TAS was determined based on a colorimetric assay, where the plasma antioxidants is known to react with ABTS (2,2'-Azino-di-[3-etylbenzthiazoline sulphate]) radical. The ABTS radical cation has a relatively stable blue-green color, which measured at 600 nm. Changes in the plasma TAS for all experimental groups after four weeks of CO oils supplementation were calculated as percentages (%) of changes.

Catalase activity

CAT activity was determined based on peroxidatic function of CAT. This assay was performed using an ELISA kit based on a protocol given by the manufacturer (Roche Diagnostics, Kuala Lumpur, Malaysia). This method is based on the enzymatic reaction with methanol in the presence of an optimal concentration of hydrogen peroxide. The formaldehyde produced was measured colorimetrically with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (purpald) as the chromogen. Purpald forms a bicyclic heterocycle with aldehyde, which upon oxidation changes from colorless to purple. Changes in the plasma CAT activities for all experimental groups after four weeks of CO oils supplementation were calculated as percentages (%) of changes.

Hepatotoxicity

The function of liver enzymes is to maintain a variety of chemical and metabolic processes that occur in the liver. In this study, ALT, AST, and GGT enzymes were used to monitor liver toxicity caused by the treatments. All the enzymes were measured enzymatically using commercial kits (Roche, Kuala Lumpur, Malaysia) on a clinical chemistry analyzer (Hitachi 902, Japan). Changes in the plasma ALT, AST, and GGT activities for all experimental groups after four weeks of CO oils supplementation were calculated as percentages (%) of changes.

Statistical analysis

Data were presented as percentages of change after four weeks of CO oils supplementation. SPSS (Statistical Package Social Sciences) version 16.0 (SPSS Corporation, Illinois, USA) for Windows was used to analyze the data. Differences in group means were determined using one-way analysis of variance (ANOVA). Tukey's pos hoc test was used for multiple group comparisons. Significance value was set at $p < 0.05$.

Results and Discussions

Food intake and body weight

Daily observation of animals recorded that rabbits remained healthy and active throughout the experiments. After two weeks of acclimatization, there were no significant changes in the body weights of all experimental groups. Baseline body weights of experimental rabbits ranged from 1.74-1.87 kg. The body weight increased significantly to 2.01-2.29 kg at week four of the study, especially in HC and HK groups. The body weight of rabbits from HC were significantly increased compared to baseline, but not for NC group.

Among the normal diet rabbit groups (NC, NP, and NK), no significant differences ($p \geq 0.05$) were found in the food intake between NC and NK. Food intake of NP group was significantly higher ($p < 0.05$) compared to the NK group (Figure 1).

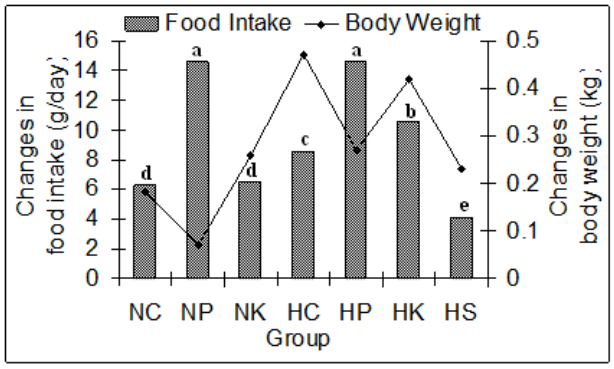

Figure 1. Changes in average food intake (g/day) and body weight (kg) after four weeks of CO pulp and kernel oils supplementations in normocholesterolemic and hypercholesterolemic rabbits

However, NK had the highest increase in body weight compared to NP and NC. There were also no significant differences ($p \geq 0.05$) in body weight changes between the treatments (NP and NK) and NC (Figure 1). For hypercholesterolemic rabbits, significant differences were found for the food intake between HC and all the treatment groups (HP, HK, and HS). The body weights of all hypercholesterolemic rabbits were not significantly differed among the hypercholesterolemic groups after four weeks of CO oils supplementation. As expected, the food intake for HP group was significantly higher than HK group after four weeks of CO oils supplementation.

Food intake of the rabbits was significantly higher in the NP group as compared to NK suggesting that the pulp oil may induce satiety of the healthy rabbits. The increased food intake would result in an increment of body weight; however, NP had lower body weight changes compared to NK. For hypercholesterolemic rabbits, a significant increase

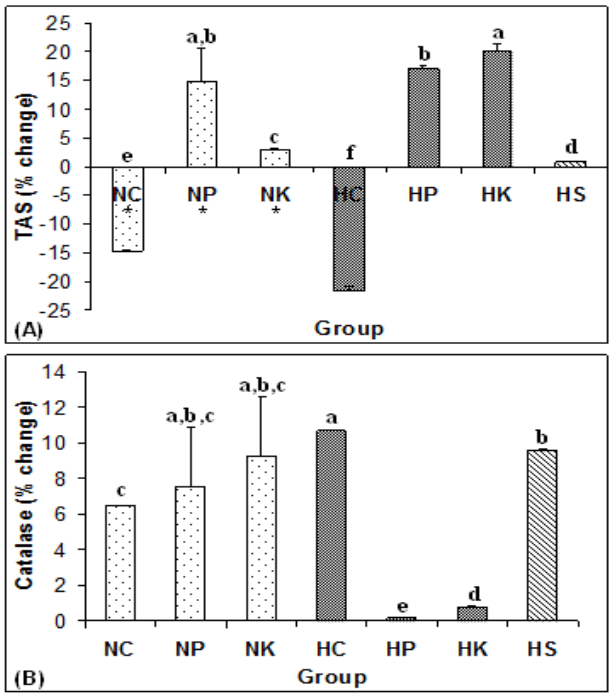

Figure 2. Changes (%) in (A) total antioxidant status (TAS) and (B) catalase activity after four weeks of CO pulp and kernel oils supplementations in normocholesterolemic and hypercholesterolemic rabbits

*The results were obtained from part of the observation that reported by Shakrin *et al*. (2012b).

in food intake was found for HP group, which shows a similar trend as NP group of normal diet rabbits. The body weight of all animals increased concomitantly with the increased of food intake.

The high body weight of animals receiving a high cholesterol diet (HC) might be due to lipid deposition in the animal's body (Chen *et al*., 2005). A significant increase in body weight of animals fed with a high cholesterol diet in the present study was in accordance with a previous study by Lee *et al.* (2006). A previous study reported that CO kernel oil has higher saturated fatty acid (60.84%) than the pulp oil (44.43%) (Azlan *et al.*, 2010). Consumption of saturated fat may increase body weight. Studies have demonstrated a linear correlation between fat saturation and increase in body weight (Rolls and Shide, 1992; Astrup *et al*., 2000). Besides, Tzang *et al*. (2009) showed that consumption of saturated fat (coconut oil and butter) could increase body weight in animals as compared to unsaturated fat (flaxseed).

Protective effects of CO oils

In this study, a protective effect of CO oils supplementation in hypercholesterolemic against oxidative stress was determined based on TAS and CAT activities. The changes of plasma TAS and CAT activities (%) of all experimental groups are shown in Figure 2. At week 0, no significant differences in plasma TAS (0.712–0.906 nmol/min/ml) were observed for all experimental rabbits, except for HS and HK groups, where both groups had significantly lower and higher plasma TAS, respectively. After four weeks of CO pulp and kernel oils supplementation to the normocholesterolemic (NP and NK) and hypercholesterolemic (HP and HK) rabbits, TAS of these rabbits were significantly improved compared to NC and HC (Figure 2A). While statin group (HS), only showed minor increased in TAS. It shows that antioxidant-rich CO oils have successfully improved the plasma TAS of the experimental rabbits in both normal and high stress conditions. In normal diet rabbits, antioxidants from the CO pulp oil have greatly contributed to the increment of plasma TAS compared with the CO kernel oil. Contrary to our expectation, the CO kernel oils had significantly increased the plasma TAS of hypercholesterolemic rabbits compared with the CO pulp oil. The reason is unknown. It is possibly that the CO kernel oil contains potent phenolic compounds able to scavenge free radicals in high stress condition.

At week 0, no significant differences in plasma CAT activity (0.1119-0.1162 nmol/min/ml) were observed for all experimental rabbits. The changes in plasma CAT activity of the experimental rabbits after four weeks of CO pulp and kernel oils supplementation are shown in Figure 2B. After four weeks of supplementation, rabbits from the hypercholesterolemic groups showed significantly increased in plasma CAT activities, except for HP and HK groups. The increment of plasma CAT activities in the HP and HK groups was observed at <1%, where the plasma CAT activity increased slightly higher in HK as compared to HP. For normal diet fed rabbits, there were high increment in plasma CAT activities of NP and NK groups. However, no significant difference in the plasma CAT activity ($p \geq 0.05$) was found between the supplementation and control groups.

In this study, a significant increased in plasma CAT activity was obvious in NC and HC. It shows that the rabbits receiving 0.5% of cholesterol daily for four weeks were having high oxidative stress condition. Conversely, Irizar and Ioannides (1998) reported that 1% cholesterol given to healthy rabbits showed no effect on CAT activity. The results show that CO oils helped to reduce CAT activity in the hypercholesterolemic groups, but not for the normal diet groups. As a result, the CO oils possibly reduce the chance of hepatocyte's susceptibility to injury (Yesilova *et al.*, 2005). It is because the oils significantly reduced the catalase activity in high oxidative stress condition (hypercholesterolemic

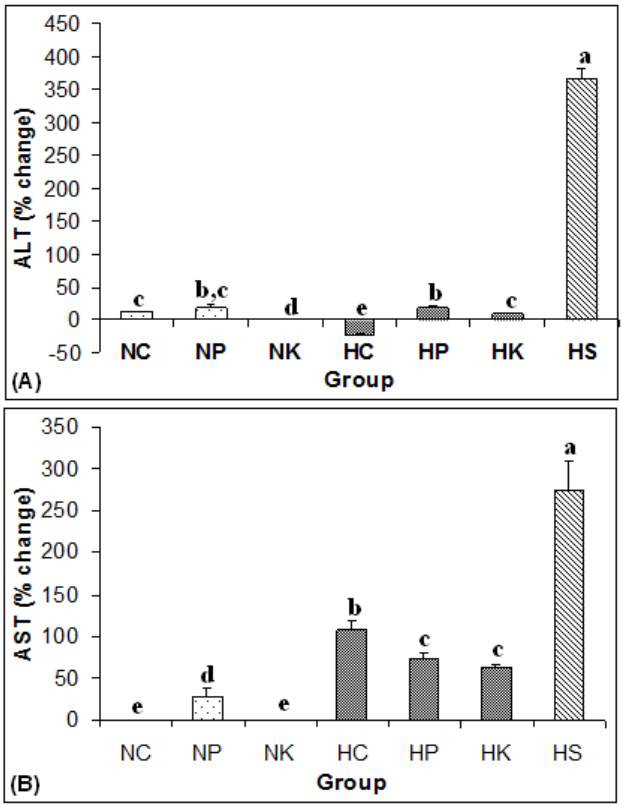

Figure 3. Changes (%) in (A) ALT and (B) AST activities after four weeks of CO pulp and kernel oils supplementations in normocholesterolemic and hypercholesterolemic rabbits

rabbits). However, in normal metabolic condition, CO oils showed no hepatoprotective effect.

Previous study found that superoxide dismutase (SOD) and glutathione peroxidase (GPx) also constitute a mutually supportive team of enzymes that protect the tissue from damage by free radicals (Heistad *et al*., 2009). Shakirin *et al.* (2012a) reported that, for normal diet rabbits supplemented with defatted dabai oils, higher SOD and GPx activities indicated the protective effects of the CO pulp and kernel oils against oxidative damages. The authors also reported significantly higher levels of plasma TAS were found for the CO oils supplemented groups of normocholesterolemic rabbits compared with control rabbits. Dietary lipids can also influence the level of the activities of these antioxidant enzymes. An increase in the activity or expression of the enzymes may protect nucleic acids, amino acid side chains in proteins and the double bonds in unsaturated fatty acids from damage by free radicals (Albertini *et al*., 2002).

Liver function tests

Changes in percentages of ALT, AST, and GGT enzymes for the normal rabbits supplemented with CO oils are shown in Figures 3 and 4, respectively. Supplementation with pulp oil for four weeks in normal diet rabbits resulted in a significant increased ($p < 0.05$) of AST and GGT (0.3 and 1.6 times higher, respectively) compared to baseline while slight increment was found for ALT. Kernel oil supplementation for four weeks had caused a significant reduction ($p < 0.05$) of AST activity and a slight reduction in ALT activity as compared to baseline. However, there was no significant change ($p \geq 0.05$) for the GGT activity between NP and NK groups.

In normal diet rabbits, the level of plasma GGT is commonly higher than the levels of ALT and AST, and supplementation of plant extracts may somehow increase the activity of ALT, AST, and GGT (Usha *et al.*, 2007). As NP rabbits have significant increased in AST activity, it shows that CO pulp oil was less beneficial to heart health as compared to CO kernel oil. Kobayashi *et al.* (2009) suggested that less than three times increment in liver function enzyme activity is considered mild and not accompanied by any hepatotoxicity. Therefore, we concluded that both pulp and kernel oils supplementation to the healthy rabbits caused no toxicity to the liver. Significant increased in the percentages of GGT changes for HP and HK groups showed that CO oil supplementation might increase the load of burden to kidney.

Liver function tests using ALT, AST, and GGT are useful markers that widely used. These are sensitive markers for possible tissue damage, particularly liver toxicity (Whitfield, 2001; Gupta *et al.*, 2005). Increase AST and ALT activities are commonly found in heart and liver diseases, where AST increment is more prominent in heart disease while ALT is higher in liver disease (Gupta *et al*., 2005). AST is an enzyme found in organs such as muscle, kidney, heart and pancreas. It is released into the blood in high quantity when there is tissue damage to the heart or liver. ALT is found primarily in the liver, where elevation of ALT activity is an indication for liver damage (Al-Mamary *et al.*, 2000), and GGT activity is related to kidney damage (Leeuwenburgh and Ji, 1995).

Changes in percentages of ALT, AST, and GGT for hypercholesterolemic rabbits after four weeks supplementation of CO oils and statin are shown in Figures 3 and 4, respectively. After four weeks of CO oils supplementation, AST activities were significantly reduced in treatment groups compared to HC and HS rabbits. HC rabbits had reduced levels of ALT and GGT activities after four weeks of high cholesterol diet. Both HP and HK rabbits had increased level in ALT activities, where HP rabbits showed a significantly higher percentage of ALT than HK rabbits (Figure 3A). GGT activity in HP rabbits was slightly decreased while HK had a

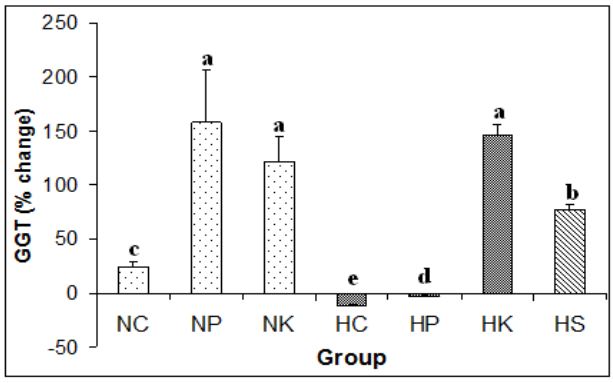

Figure 4. Changes (%) in GGT activity after four weeks of CO pulp and kernel oils supplementations in normocholesterolemic and hypercholesterolemic rabbits

significantly increased in GGT activity (Figure 4). The percentages of change in AST activity for HP and HK were not significantly differed. ALT, AST, and GGT activities of HS rabbits were significantly increased as compared to the hypercholesterolemic rabbits supplemented with CO oils and control, except percentage of GGT for HK. The reason for the significant high GGT activity in HK rabbits is not known.

As HS rabbits had high ALT, AST, and GGT activities, simvastatin as treatment was shown to induce hepatotoxicity. Plasma ALT exhibited the greatest increased (>19 times) in the HS group (Figure 3A). The high ALT activity in the statin group is related to liver damage (Al-Mamary *et al.*, 2002) because statin is toxic to liver. Björnsson *et al.* (2012) reported that simvastatin is commonly caused hepatocellular injury while atorvastatin is highly associated with cholestatic liver injury. They also revealed higher levels of ALT and AST were found for simvastatin treated patients compared with atorvastatin treatment. A high percentage of AST increased in HS also shows that statin is highly toxic to other organs besides the liver. As in hypercholesterolemic rabbits, CO pulp oil supplementation had improved the kidney function as compared to normal diet rabbits while CO kernel oil showed no improvement in kidney function that indicated by higher increment of percentage of GGT. A high increased in percentage of GGT in HS rabbits also show that statin caused a great burden to kidney too.

One of the main functions of liver is to detoxify toxins from the body. In order to evaluate hepatotoxicity, liver function tests were carried out to evaluate hepatocyte injuries and to assess the liver function. ALT, AST, and GGT are the most commonly-used markers of hepatocyte injuries (Whitfield, 2001; Mandal *et al.*, 2002). Abnormal values in liver function test reflect hepatocellular damage or dysfunction (Al-Mamary *et al.*, 2002). Mehendale *et al. (*1994) also reported high serum transaminase, particularly ALT, caused by increased membrane permeability of the hepatocyte and the release of enzymes from liver cells into serum.

In this study, when feeding the animals with high cholesterol diet, AST and GGT levels were significantly elevated. These results were supported by Souza *et al.* (2010). However, feeding a high cholesterol diet to rabbits for the same duration by Prasad (2008) did not produce any significant elevation of plasma AST and GGT. It could be due to the lower level of cholesterol (0.25%) given. Hypercholesterolemia is possibly associated with hepatotoxicity, where a remarkable increase of plasma liver enzymes was noted in this study. It is related to toxicity and the peroxidation of lipids in the serum and tissues. This observation is also well described by previous studies (Mahmoud *et al*., 2002; Shin *et al.*, 2007). ALT is a cytosolic enzyme in the liver that differs from AST. It has more mitochondrial enzyme (approximately 80%) (Al-Mamary *et al*., 2002). However, AST is not released as fast as ALT.

Previous studies reported that the 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase inhibitor (statin) is a well-established class of drug in the treatment of hypercholesterolemia. The members of the class have been shown to reduce the risk of cardiovascular morbidity and mortality in patients with or at risk for coronary heart disease in several clinical trials (Shin *et al*., 2007; Kolovou *et al*., 2008). The most important adverse effects associated with statins (atorvastatin, fluvastatin, lovastatin, pravastatin, rosuvastatin, and simvastatin) are asymptomatic increases in liver transaminases and myopathy (Bellosta *et al*., 2004). It seems very clear that the treatment for CVD-related diseases using statins has side effects. Therefore, antioxidant-rich CO oils are the best choice to replace the use of statin in prevention of CVD. The oils are also safe for consumption even in a large amount.

Conclusion

Pulp oil is considered safe as the increment of plasma toxicities (ALT, AST, and GGT) were considered mild. Similarly to the pulp oil of CO, no toxic effect was found for the consumption of kernel oil because plasma AST and ALT levels were significantly reduced in the treatment groups as compared to the statin group. The activities of plasma AST and GGT in these groups were found to be significantly higher compared to animals fed a normal diet. Feeding a high cholesterol diet resulted no significant change of CAT activity. The

administration of CO oils to rabbits fed with high cholesterol diet for four weeks showed no sign of hepatotoxicity as there was a reduction in liver function indicators (AST and GGT). The study also showed that administration of simvastatin induced hepatotoxicity as there was a significant elevation in liver function tests (ALT, AST, and GGT).

Acknowledgement

The study was supported by the Research University Grant Scheme (RUGS), Universiti Putra Malaysia (Vote No. 91059).

References

Agunbiade, S. O. and Olanlokun, J. O. 2006. Evaluation of some nutritional characteristics of Indian almond *(Prunus amygdalus)* nut. Pakistan Journal of Nutrition 5(4): 316-318.

Al-Mamary, M., Al-Habori, M., Al-Aghbari, A. M. and Baker, M. M. 2002. Investigation into the toxicological effects of *Catha edulis* leaves: a short term study in animals. Phytotherapy Research 16(2): 127-132.

Albertini, R., Moratti, R. and de Luca, G. 2002. Oxidation of low-density lipoprotein in atherosclerosis from basic biochemistry to clinical studies. Current Molecular Medicine 2(6): 579-592.

Astrup, A., Ryan, L., Grunwald, G. K., Storgaard, M., Saris, W., Melanson, E. and Hill, J. O. 2000. The role of dietary fat in body fatness: evidence from a preliminary meta-analysis of ad libitum low-fat dietary intervention studies. British Journal of Nutrition 83(S1): S25–S32.

Azlan, A., Prasad, K. N., Khoo, H. E., Abdul-Aziz, N., Mohamad, A., Ismail, A. and Amom, Z. 2010. Comparison of fatty acids, vitamin E and physicochemical properties of *Canarium odontophyllum* Miq. (dabai), olive and palm oils. Journal of Food Composition and Analysis 23(8): 772-776.

Basu, M., Prasad, R., Jayamurthy, P., Pal, K., Arumughan, C. and Sawhney, R. C. 2007. Anti-atherogenic effects of seabuckthorn *(Hippophaea rhamnoides)* seed oil. Phytomedicine 14(11): 770-777.

Bellosta, S., Paoletti, R. and Corsini, A. 2004. Safety of statins focus on clinical pharmacokinetics and drug interactions. Circulation 109(Suppl III): III-50–III-57.

Björnsson, E., Jacobsen, E. I. and Kalaitzakis, E. 2012. Hepatotoxicity associated with statins: reports of idiosyncratic liver injury post-marketing. Journal of Hepatology 56(2): 374-380.

Carter, C., Finley, W., Fry, J., Jackson, D. and Willis, L. 2007. Palm oil markets and future supply. European Journal of Lipid Science and Technology 109(4): 307-314.

Chen, C. C., Liu, L. K., Hsu, J. D., Huang, H. P., Yang, M. Y. and Wang, C. J. 2005. Mulberry extract inhibits the development of atherosclerosis in cholesterol-fed rabbits. Food Chemistry 91(4): 601-607.

Chew, L. Y., Prasad, K. N., Amin, I., Azrina, A. and Lau, C. Y. 2011. Nutritional composition and antioxidant properties of *Canarium odontophyllum* Miq. (dabai) fruits. Journal of Food Composition and Analysis 24(4): 670-677.

Covas, M. I., Nyyssönen, K., Poulsen, H. E., Kaikkonen, J., Zunft, H. J. F., Kiesewetter, H., Gaddi, A., de la Torre, R., Mursu, J., Bäumler, H., Nascetti, S., Salonen, J., Fitó, M., Virtanen, J., Marrugat, J. and EUROLIVE Study Group. 2006. The effect of polyphenols in olive oil on heart disease risk factors: A randomized trial. Annals of Internal Medicine 145(5): 333-341.

de Souza, M. O., Silva, M., Silva, M. E., de Paula Oliveira, R. and Pedrosa, M. L. 2010. Diet supplementation with acai (*Euterpe oleracea* Mart.) pulp improves biomarkers of oxidative stress and the serum lipid profile in rats. Nutrition 26(7): 804-810.

Gonzalez-Santiago, M., Martin-Baustist,a E., Carrero, J. J., Fonollá, J., Baró, L., Bartolomé, M. V., Gil-Loyzaga, P. and López-Huertas, E. 2006. One-month administration of hydroxytyrosol, a phenolic antioxidant present in olive oil, to hyperlipidemic rabbits improves blood lipid profile, antioxidant status and reduces atherosclerosis development. Atherosclerosis 188(1): 35-42.

Gupta, R. K., Kesari, A. N., Watal, G., Murthy, P. S., Chandra, R. and Tandon, V. 2005. Nutritional and hypoglycemic effect of fruit pulp of *Annona squamosa* in normal healthy and alloxan-induced diabetic rabbits. Annals of Nutrition and Metabolism 49(6): 407-413.

Heistad, D. D., Wakisaka, Y., Miller, J., Chu, Y. and Pena-Silva, R. 2009. Novel aspects of oxidative stress in cardiovascular diseases. Circulation Journal 73(2): 201-207.

Hu, F. B. 2003. Plant-based foods and prevention of cardiovascular disease: an overview. American Journal of Clinical Nutrition 78(3): 544S-551S.

Irizar, A. and Ioannides, C. 1998. Marked inhibition of hepatic cytochrome P450 activity in cholesterol-induced atherosclerosis in rabbits. Toxicology 126(3): 179-193.

Knio, K. M., Usta, J., Dagher, S., Zournajian, H. and Kreydiyyeh, S. 2008. Larvicidal activity of essential oils extracted from commonly used herbs in Lebanon against the seaside mosquito, *Ochlerotatus caspius*. Bioresource Technology 99(4): 763-768.

Kobayashi, A., Suzuki, Y., Kuno, H., Sugai, S., Sakakibara, H. and Shimoi, K. 2009. Effects of fenofibrate on plasma and hepatic transaminase activities and hepatic transaminase gene expression in rats. Journal of Toxicological Sciences 34(4): 377-387.

Kolovou, G. D., Vasiliadis, I., Anagnostopoulou, K. and Cokkinos, D. V. 2008. Simvastatin: two decades in a circle. Cardiovascular Therapeutics 26(2): 166-178.

Lee, H. S., Ahn, H. C. and Ku, S. K. 2006. Hypolipemic effect of water extracts of *Picrorrhiza rhizoma* in PX-407 induced hyperlipemic ICR mouse model with hepatoprotective effects: A prevention study. Journal

of Ethnopharmacology 105(3): 380-386.

Leeuwenburgh, C. and Ji, L. L. 1995. Glutathione depletion in rested and exercised mice: biochemical consequence and adaptation. Archives of Biochemistry and Biophysics 316(2): 941-949.

Lim, T. K. 2013. Edible medicinal and non-medicinal plant. Fruits, 5th vol. Dordrecht, Heidelberg, London, New York: Springer.

Mahmoud, M. R., El-Abhar, H. S. and Saleh, S. 2002. The effect of *Nigella sativa* oil against the liver damage induced by *Schistosoma mansoni* infection in mice. Journal of Ethnopharmacology 79(1): 1-11.

Mandal, A. K., Sinha, J., Mandal, S., Mukhopadhyay, S. and Das, N. 2002. Targeting of liposomal flavonoid to liver in combating hepatocellular oxidative damage. Drug Delivery 9(3): 181-185.

Mehendale, H. M., Thakore, K. N. and Rao, C. V. 1994. Autoprotection: stimulated tissue repair permits recovery from injury. Journal of Biochemical Toxicology 9(3): 131-139.

Nyong, B. E., Edem, C. A., Obochi, G. O. and Malu, S. P. 2009. Effect of methods of extraction on phytochemical constituents and antibacterial properties of *Tetracarpidium conophorum* seeds. Global Journal of Pure and Applied Sciences 15(3-4): 373-376.

Pokorný, J. 1991. Natural antioxidants for food use. Trends in Food Science and Technology 2: 223-227.

Prasad K. 2010. Effects of vitamin E on serum enzymes and electrolytes in hypercholesterolemia. Molecular and Cellular Biochemistry 335(1-2): 67-74.

Prasad, K. 2008. Serum biochemical changes in rabbits on a regular diet with and without flax lignan complex following a high-cholesterol diet. International Journal of Angiology 17(1): 27-32.

Rolls, B. J. and Shide, D. J. 1992. The influence of dietary fat on food intake and body weight. Nutrition Reviews 50(10): 283-290.

Shakirin, F. H., Azlan, A., Ismail, A., Amom, Z. and Lau, C. Y. 2012a. Anti-atherosclerotic effect of *Canarium odontophyllum* Miq. fruit parts in rabbits fed high cholesterol diet. Evidence-Based Complementary and Alternative Medicine 2012(Art 838604): 1-10.

Shakirin, F. H., Azlan, A., Ismail, A., Amom, Z. and Lau, C. Y. 2012b. Protective effect of pulp oil extracted from *Canarium odontophyllum* Miq. fruit on blood lipids, lipid peroxidation, and antioxidant status in healthy rabbits. Oxidative Medicine and Cellular Longevity 2012(Art 840973): 1-9.

Shimizu, T., Nakai, K., Morimoto, Y., Ishihara, M., Oishi, H., Kikuchi, M. and Arai, H. 2009. Simple rabbit model of vulnerable atherosclerotic plaque. Neurologia Medico-Chirurgica 49(8): 327-332.

Shin, M. J., Chung, N., Lee, J. H., Jang, Y., Park, E., Jeon, K. I., Chung, J. H. and Seo, B. Y. 2007. Effects of simvastatin on plasma antioxidant status and vitamins in hypercholesterolemic patients. International Journal of Cardiology 118(2): 173-177.

Tzang, B. S., Yang, S. F., Fu, S. G., Yang, H. C., Sun, H. L. and Chen, Y. C. 2009. Effects of dietary flaxseed oil on cholesterol metabolism of hamsters. Food Chemistry 114(4): 1450-1455.

Usha, K., Kasturi, G. M. and Hemalatha, P. 2007. Hepatoprotective effect of *Hygrophila spinosa* and *Cassia occidentalis* on carbon tetrachloride induced liver damage in experimental rats. Indian Journal of Clinical Biochemistry 22(2): 132-135.

Whitfield, J. B. 2001. Gamma glutamyl transferase. Critical Reviews in Clinical Laboratory Sciences 38(4): 263-355.

Yesilova, Z., Yaman, H., Oktenli, C., Ozcan, A., Uygun, A., Cakir, E., Sanisoglu, S. Y., Erdil, A., Ates, Y., Aslan, M., Musabak, U., Erbil, K., Karaeren, N. and Dagalp, K. 2005. Systemic markers of lipid peroxidation and antioxidants in patients with nonalcoholic fatty liver disease. American Journal of Gastroenterology 100(4): 850-855.

Zhao, S., Chu, Y., Zhang, C., Lin, Y., Xu, K., Yang, P., Fan, J. and Liu, E. 2008. Diet-induced central obesity and insulin resistance in rabbits. Journal of Animal Physiology and Animal Nutrition 92(1): 105-111.