Durian (*Durio zibethinus Murr.*) cultivars as nutritional supplementation to rat's diets

Hanna Leontowicz [a], Maria Leontowicz [a], Ratiporn Haruenkit [b], Sumitra Poovarodom [c], Zenon Jastrzebski [d], Jerzy Drzewiecki [e], Alma Leticia Martinez Ayala [f], Iwona Jesion [a], Simon Trakhtenberg [g], Shela Gorinstein [h,1,*]

[a] *Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw Agricultural University, Warsaw, Poland*
[b] *Faculty of Agricultural Industry, King Mongkut's Institute of Technology Ladkrabang, Ladkrabang, Bangkok 10520, Thailand*
[c] *Department of Soil Science, King Mongkut's Institute of Technology Ladkrabang, Ladkrabang, Bangkok 10520, Thailand*
[d] *Department of Pharmacology, National Institute of Public Health, Poland*
[e] *Plant Breeding and Acclimatization Institute, Radzikow, Poland*
[f] *Departamento De Biotecnología, Centro de Desarrollo de Productos Bioticos, Instituto Politecnico Nacional, Morelos, Mexico*
[g] *Kaplan University Medical Center, Rehovot, Israel*
[h] *Department of Medicinal Chemistry and Natural Products, School of Pharmacy, The Hebrew University – Hadassah Medical School, Jerusalem, Israel*

Abstract

The properties of Mon Thong, Chani and Kan Yao durian (*Durio zibethinus Murr.*) cultivars were compared *in vitro* and *in vivo* studies in order to find the best one as a supplement to antiatherosclerotic diet. Total polyphenols (361.4 ± 35.3 mgGAE/100 g FW), flavonoids (93.9 ± 8.9 mgCE/100 g FW) and total antioxidant capacity determined by DPPH and β-carotene-linoleic acid assays (261.3 ± 25.3 μMTE/100 g FW and $77.8 \pm 7.8\%$ of inhibition) were maximal in Mon Thong in comparison with Chani and Kan Yao and showed a good correlation between these three variables ($R^2 = 0.9859$).

Five groups of rats were fed diets supplemented with cholesterol and different durian cultivars. Diets supplemented with Mon Thong and to a lesser degree with Chani and Kan Yao significantly hindered the rise in the plasma lipids (TC – 8.7%, 16.1% and 10.3% and (b) LDL-C – 20.1%, 31.3% and 23.5% for the Chol/Kan Yao, Chol/Mon Thong and Chol/Chani, respectively) and the decrease in plasma antioxidant activity ($P < 0.05$).

Nitrogen retention remained significantly higher in Chol/Mon Thong than in other diet groups. Diet supplemented with Mon Thong affected the composition of plasma fibrinogen in rats and showed more intensity in protein bands around 47 kDa. No lesions were found in the examined tissue of heart and brains. Mon Thong cultivar is preferable for the supplementation of the diet as positively influenced the lipid, antioxidant, protein and metabolic status. The durian fruit till now was not investigated extensively, therefore based on the results of this study durian cultivars can be used as a relatively new source of antioxidants.
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1. Introduction

The protective effects of natural products are considered, in large part, to be related to the content of various substances: phenolic compounds and to less extend dietary fiber (Martinez-Gonzalez et al., 2002; Suksomtip et al., 2004; Dallongeville et al., 2006; Halvorsen et al., 2006).

Among exotic fruits durian (*Durio zibethinus Murr.*) is less known, and the differences between its cultivars is practically not studied (Ketsa and Daengkanit, 1999; Mahattanatawee et al., 2006; Leontowicz et al., 2007). The content of bioactive compounds and the influence of cultivars in the experiments on laboratory animals and in investigations of humans are significantly different (Gorinstein et al., 2005, 2006). The synergetic effect, which could exist between individual bioactive compounds, means that the antioxidant capacity may be higher than their sum (Poeggeler et al., 1995), and not only individual bioactive compounds, but also the overall antioxidant capacity have to be determined. Some antioxidant assays give different antioxidant activity trends (Ou et al., 2002).

Therefore it was decided to investigate the bioactive compounds of three cultivars of durian (Mon Thong, Kan Yao and Chani) *in vitro* and to find out how they affect the plasma lipid profile and the antioxidant capacity in rats fed cholesterol containing diets.

In order to receive reliable data two each other complemented assays for the determination of the total antioxidant capacity were used: β-carotene and 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and compared with the total polyphenols determined by Folin–Ciocalteu method.

As far as we know, there are no such investigations of supplementation of durian cultivars to the diets of rats.

2. Materials and methods

2.1. Chemicals

2, 4, 6-tripyridyl-*s*-triazine (TPTZ) was purchased from Fluka Chemie, Buchs, Switzerland. β-Carotene, Folin–Ciocalteau reagent; cholesterol of analytical grade (USP), DPPH (1,1-diphenyl-2-picrylhydrazyl); $FeCl_3 \cdot 6H_2O$; $CuCl_2 \cdot 2H_2O$, neocuproine (2, 9-dimethyl-1,10-phenanthroline), cholesterol and Trolox (6-hydroxy-2,5,7,8,-tetramethyl-chroman-2-carboxylic acid); BHA (butylated hydroxyanisole); potassium persulfate, sodium dodecyl sulfate (SDS), β-mercaptoethanol (β-ME), acrylamide, polyacrylamide, Coomassie Brilliant Blue R and molecular weight marker (14–205 kDa) were obtained from Sigma Chemical Company, St. Louis, MO, USA. All reagents were of analytical grade. Deionized and distilled water was used throughout.

2.2. Samples preparation

In this investigation Mon Thong, Kan Yao and Chani cultivars at their ripening stages were studied, using 5 replicates of five fruit each. Fruits were cleaned with tap water and dried. Harvesting and determination of maturity was carried out by Thai very skilled workers. They combined the following techniques: day count, character of fruit spines, tapping the fruit, color and shape of all fruit. The edible parts of the above-mentioned cultivars were prepared manually without using steel knives. The peeled fruits were weighed, chopped and homogenized under liquid nitrogen in a high-speed blender (Hamilton Beach Silex professional model) for one minute. Then a weighed portion (50–100 g) was lyophilized for 48 h (Virtis model 10–324) and the dry weight was determined. The samples were ground to pass through a 0.5-mm sieve and stored at −20 °C until analyzed.

2.3. Determination of the bioactive substances

Dietary fibers in the selected samples were analyzed by the modified AOAC method (Prosky et al., 1988). Samples were treated with heat-stable α-amylase, protease, and amyloglucosidase, followed by centrifugation (15 min, 3000*g*) to separate the soluble and insoluble fractions and dialysis against water.

Minerals (Na, K, Mg, Ca) and trace elements (Fe, Cu, Zn and Mn): 0.8 g of lyophilized samples was mineralized in microwave oven with concentrated HNO_3. The concentrations of above mentioned elements were estimated by a Perkin–Elmer 5100 ZL atomic absorption spectrometer (Perkin–Elmer Ltd., Beaconsfield, Buckinghamshire, England), using the flame method for Na, K, Mg, Ca, Fe, Cu, Zn and the flameless method for Mn.

Defatted lyophilized fruit samples were extracted from a 50-mg aliquot with 5 mL of 1.2 M HCl in 50% methanol/water for total polyphenols (TP) with heating at 90 °C for 3 h. The samples were cooled, diluted to 10 mL with methanol and centrifuged for 5 min at 4000*g* with a benchtop centrifuge to remove solids. The Folin–Ciocalteu method was used, and the measurement was performed at 765 nm with gallic acid as the standard. The results were expressed as mg gallic acid equivalents (GAE)/100 g FW (Singleton et al., 1999).

The absorbance of flavonoids (extracted with 5% $NaNO_2$, 10% $AlCl_3 \times 6H_2O$ and 1 M NaOH) was measured at 510 nm with the standards prepared similarly with known (+)-catechin concentrations. The results were expressed as mg of catechin equivalents (CE)/100 g FW.

2.4. Determination of the antioxidant capacity

In this study the following two methods were applied: β-Carotene-linoleic acid assay and 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) assay.

2.4.1. First method

A stock solution of β-carotene and linoleic acid was prepared by dissolving 0.5 mg of β-carotene in 1 mL of chloroform and adding 25 μL of linoleic acid together with 200 mg of Tween 40 (Ferreira et al., 2006). The chloroform was evaporated. One hundred ml of aerated water were added to the residue. To 2.5 mL of this mixture 300 μL of each extract were added. The samples were incubated in boiling water for 120 min together with two blanks, one containing the antioxidant BHA and the other one without antioxidant. The absorbance was measured at 470 nm.

2.4.2. Second method

The volume of durian extracts in different test tubes for the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) assay was adjusted to 100 μL by adding MeOH. A 0.1 mM methanolic solution of DPPH was added (5 μL) to these tubes. The control was prepared as above without any extract, and MeOH was used for the baseline correction. Changes in the sample absorbance were measured at 517 nm on an Uvikon 930 spectrometer (Kontron Instruments, Watford, UK). Butylated hydroxyanisole (BHA) was used for comparison (Singh et al., 2002).

2.5. Rats, diets and laboratory tests

The weight of the male Wistar rats ($n = 35$) at the beginning of the experiment was 111 g. The rats were divided into 5 groups each of 7, and named Control, Chol, Chol/Mon Thong, Chol/Chani and Chol/Kan Yao. During 30 days of the experiment the rats of all 5 groups were fed a basal diet (BD), which included wheat starch, casein, soybean oil, vitamin and mineral mixtures. The rats of the Control group were fed only the BD. The BD of the other 4 groups was supplemented with: 10 g/kg of nonoxidized cholesterol (NOC) of analytical grade (Chol group), 10 g/kg of NOC and 50 g/kg of Mon Thong durian cultivar (Chol/Mon Thong), 10 g/kg of NOC and 50 g/kg of Chani durian cultivar (Chol/Chani) and 10 g/kg of NOC and 50 g/kg of Kan Yao durian cultivar (Chol/Kan Yao). The cholesterol batches were mixed with the BD (1:99).

All rats were fed once a day at 10^{00}h ad libitum. They had unrestricted access to drinking water. The feed intake was monitored daily and body gains every week (Leontowicz et al., 2007).

In order to assess the nutritional value of the used diets the following indices were determined: dry matter (DM) and crude protein (CP) in the diets and their digestibility, and the influence of different diets on nitrogen retention.

For the determination of the nitrogen retention from the diets, feces and urine were collected in the last 5 days of the investigation. Samples were dried at 80 °C for 3 days and then at 105 °C for 3 additional days for estimation of the dry matter of 5 diets and feces. The nitrogen was determined by Kjeldahl (Kjeltec-300 Tecator).

At the end of the experiment the rats were anaesthetized using diethyl ether and the blood samples were taken from the left atrium of the heart. Plasma was prepared and used for laboratory tests. Two time points were used in this experiment: before and after 30 days of feeding. Total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C) and triglycerides (TG) were determined as previously described (Gorinstein et al., 2005, 2006).

For the determination of the plasma antioxidant capacity three antioxidant tests were adopted: DPPH, Ferric-reducing/antioxidant power (FRAP) and Cupric reducing antioxidant capacity (CUPRAC).

In the determination of plasma antioxidant capacity for the DPPH method 25 μL of plasma were mixed with 75 μL of MeOH and 800 μL of 75 μM DPPH. The reaction mixture was maintained in dark at room temperature for 90 min, and the absorbance at 517 nm was then recorded (Singh et al., 2002).

Ferric-reducing/antioxidant power (FRAP) assay measures the ability of the antioxidants contained in the samples to reduce ferric-tripiridyl-triazine (Fe^{3+}-TPTZ) to a ferrous form (Fe^{2+}) which absorbs light at 593 nm. The ferro- and ferric-iron form complexes with TPTZ reagent are the main products of this reaction. The antioxidant capacity of 10 μL of plasma was measured (Benzie and Strain, 1996).

Cupric reducing antioxidant capacity (CUPRAC). This assay is based on utilizing the copper (II)-neocuproine [Cu (II)-Nc] reagent as the chromogenic oxidizing agent. To the mixture of 1 mL of Cu (II), Nc, and NH_4Ac buffer solution, antioxidant sample (or standard) solution ($\times$ mL) and H_2O [(1.1 − $\times$) ml] were added to make the final volume of 4.1 mL. The absorbance at 450 nm was recorded against a reagent blank. The results of antioxidant activity were expressed in mM of trolox equivalents TE/L (Apak et al., 2006).

Serum fibrinogen was precipitated with methanol, then purified by sequential DEAE anion-exchange chromatography, dialyzed against water for 72 h, and lyophilized. Plasma samples were dissolved in sample buffer: 2% SDS; 10% glycerol, 2%-mercaptoethanol, 0.002% bromophenol blue and 0.62 M Tris HCl, pH 6.8. Electrophoresis was performed (Laemmli, 1970) with the Hoeffer SE 600 vertical unit (Hoeffer Pharmacia Biotech Inc., San Francisco, CA 94107, USA) using polyacrylamide gels (resolving gel $T = 13.7\%$, $C = 1.7\%$, stacking gel $T = 3.8\%$, $C = 1.8\%$) with gel size of $180 \times 160 \times 1.5$ mm. Sample size was 5 μL. The run was carried out at 25 mA per gel until the end of electrophoresis. Gels were stained with 0.25% Coomassie Brillant Blue R in methanol/water/glacial acetic acid (5:5:1 v/v), destained in water and scanned in transmission light with an Agfa SNAPSCAN 1236 (Agfa-Gevaert N.V Belgium, Agfa SnapScan 1236 s Color image scanner).

Histological analyses of the heart and brain samples were done after a formalin fixation. Segments of the heart and brains were processed by a common paraffin technique. Each sample was cut into 72 serial sections (thickness of 5 μm) with a transversally oriented cutting plane, and stained with hematoxylin and eosin and green trichrome (Bobková and Tonar, 2005). The Animal Care Committee of the Warsaw Agricultural University, Poland, had approved this study.

2.6. Statistical analyses

The results of this investigation *in vitro* are means ± SD of five measurements. Differences between groups were tested by two-way ANOVA. In the assessment of the antioxidant potential, Spearman correlation coefficient (R) was used. Linear regressions were also calculated. The *p* values of <0.05 were considered significant.

3. Results

3.1. In vitro experiments

It was found that the dietary fibers and the minerals (Na, K, Mg and Ca) and the trace elements (Fe, Mn, Zn and Cu) were comparable in all three cultivars ($P > 0.05$).

Soluble dietary fiber was found in the range of 0.35–0.40 g/100 g FW; minerals (mg/100 g fresh fruit) varied from: (Na from 2.6 to 5.7); (K from 91.1 to 151.3); (Mg from 4.5 to 8.4); (Ca from 3.5 to 8.1). The contents of trace elements in durian cultivars (μg/100 g fresh weight) were in the following order: (Fe from 110 to 150); (Mn from 12.1 to 19.3); (Zn from 49.5 to 78.4) and (Cu from 39.5 to 63.1). Potassium had the highest content among the minerals and Fe – among the trace elements. According to their contents minerals and trace element showed the following order: K > Mg > Ca > Na and of the trace elements – Fe > Zn > Cu > Mn, respectively.

Total polyphenols and flavonoids were maximal in the Mon Thong in comparison with the Chani and Kan Yao samples (Fig. 1a and b). The DPPH, β-carotene and Folin–Ciocalteu assays showed a significant increase ($P < 0.05$ in all cases) in the antioxidant capacities and in
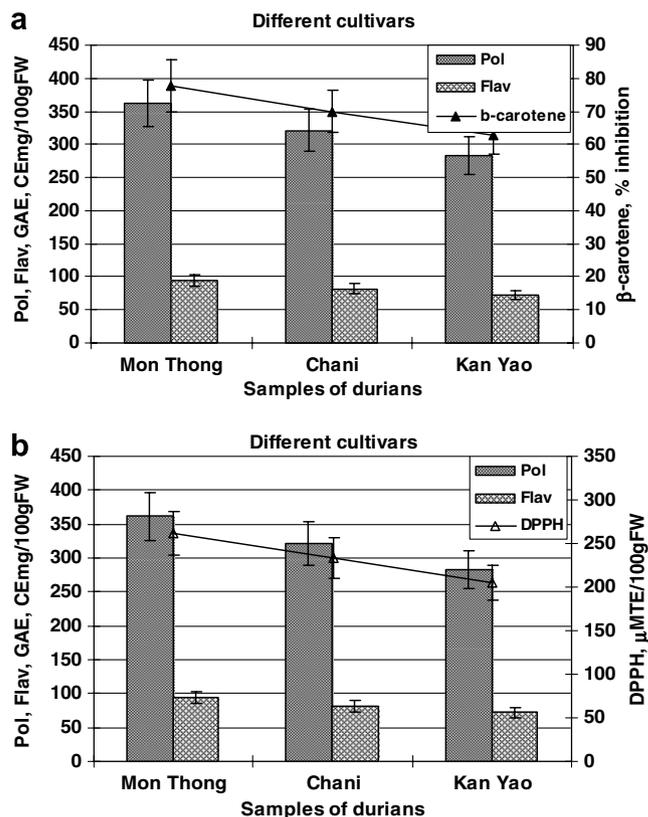

Fig. 1. Antioxidant capacities determined by some radical scavenging assays of different durian cultivars (Mon Thong, Shani, Kan Yao). A, Pol, polyphenols, mgGAE/ 100 g FW, Flav, flavonoids, mgCE/100 g FW; and antioxidant capacity by β-carotene, % inhibition. B, Pol, polyphenols, mgGAE/ 100 g FW, Flav, flavonoids, mgCE/100 g FW; and antioxidant capacity by 1, 1-diphenyl-2 -picrylhydrazyl (DPPH), μMTE/100 g FW.

the content of total polyphenols in Mon Thong and Chani samples (Fig. 1a and b). A very good correlation was observed (Fig. 2a) between the antioxidant activity determined by DPPH and the total polyphenols ($R^2 = 0.9859$) and slightly lower one was calculated for β-carotene assay ($R^2 = 0.812$). Flavonoids showed lower correlation coefficients (Fig. 2b) than the total polyphenols ($R^2 = 0.9298$; 0.7759) and their antioxidant activities.

3.2. In vivo experiments

The feed intake (g/day) and the protein efficiency ratio in the Mon Thong and Chani diet groups were significantly higher than in three other groups ($P < 0.05$) and were expressed as 16.67 ± 1.36 and 16.37 ± 1.03 and 0.45 ± 0.03 and 0.44 ± 0.04, respectively. These data were significantly higher than in three other groups ($P < 0.05$). Opposite, the body gains and feed ratio in all five groups were comparable ($P > 0.05$).

The content of crude protein (CP) in the diets of the Mon Thong and Chani diet groups was significantly higher than in others ($P < 0.05$). The dry matter and the digestibility did not show significant changes (Fig. 3).

Nitrogen retention remains significantly higher (Fig. 4) in the Chol/Mon Thong and to a less degree in Chol/Chani than in other groups (0.23 ± 0.03 and 0.205 ± 0.03 g/day, 62% and 54%, respectively, P in both cases <0.05). It can

Fig. 3. Dry matter (DM) and crude protein (CP) in the diets and their digestibility as determined at the end of the experiment (in %). Values are means ± SD ($n = 7$). Values with different superscription letters in columns are significantly different ($P < 0.05$). Abbreviations: Digest, digestibility.
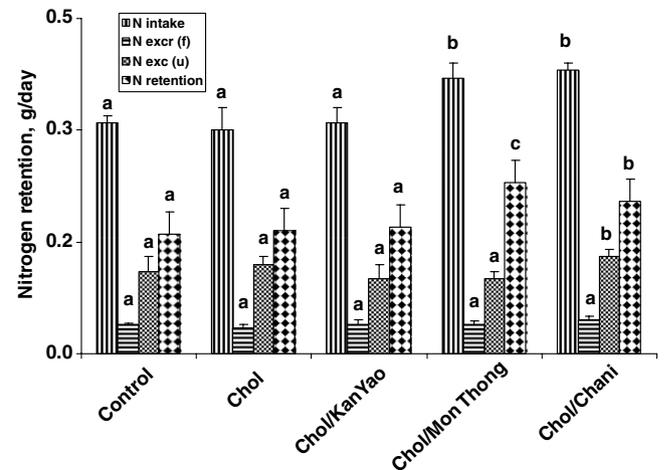
Fig. 4. The influence of the different diets on nitrogen retention (g/day). Values are means ± SD ($n = 7$). Values with different superscription letters in columns are significantly different ($P < 0.05$). Abbreviations: Chol, cholesterol diet group; Chol/Chani, diet group supplemented with durian Chani; Chol/Mon Thong, diet group supplemented with durian Mon Thong; Chol/Kan Yao, diet group supplemented with durian Kan Yao; excr, excretion; f, feces; n, nitrogen; u, urine.

be explained by the high nitrogen intake and relatively low nitrogen excretion in feces and urine in these diet groups.

Durian cultivars supplemented diets significantly hindered the rise of plasma total cholesterol vs. Chol diet group (Fig. 5a):

(a) TC – 8.7%, 16.1% and 10.3% and (b) LDL-C – 20.1%, 31.3% and 23.5% for the Chol/Kan Yao, Chol/Mon Thong and Chol/Chani, respectively.

A decrease in the plasma antioxidant capacity values as determined by three each other supplemented antioxidant
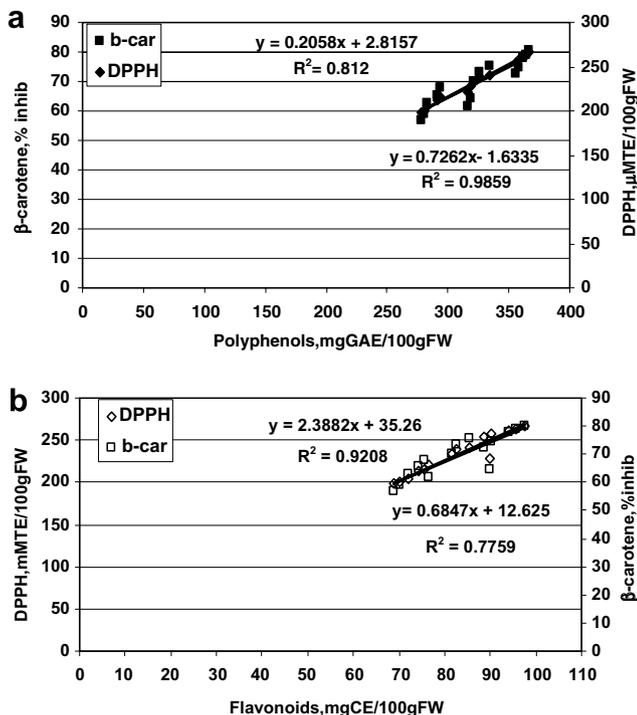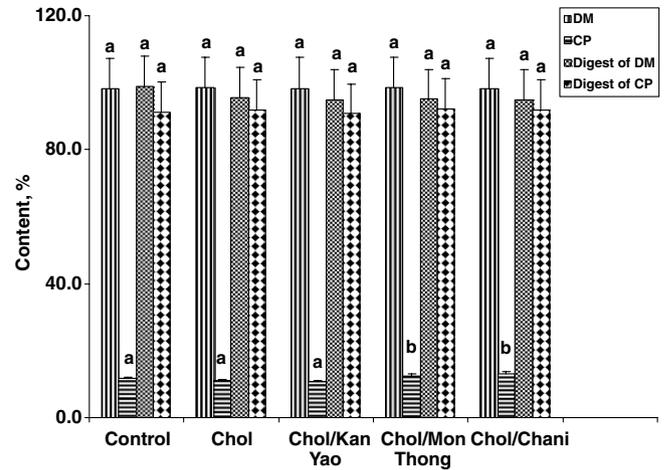

Fig. 2. Relationship, calculated by a linear regression analysis for durian cultivars and their antioxidant capacities. A, (■) Polyphenols (mgGAE/100 g, X) and β-carotene (% inhibition, Y_1) to (◆) Polyphenols (mgGAE/100 g, X) and 1, 1-diphenyl-2 -picrylhydrazyl (DPPH) (μmol/100 g, Y_2). B, (◇) Flavonoids (mgCE/100 g FW) and DPPH (μmol/100 g FW, Y_1) to (□) Flavonoids (mgCE/100 g FW) and β-carotene (% inhibition, Y_2).

Fig. 6. Comparison of the band intensity of serum proteins after different durian diets extracted with sample buffer containing SDS and 2-ME and separated by SDS-PAGE. Molecular markers (kDa): 205-myosin; 116-β-galactosidase; 97-phosphorylase b; 66-albumin; 45-ovalbumin; 36-glyceralaldehyde-3-phosphate dehydrogenase; 29-carbonic anhydrase; 24-trypsinogen, PMSF treated; 20-trypsin inhibitor, 14-α-lactalbumin; loading 2 µl: Con, control; Chol, cholesterol; DMT, DMTch, durian Mon Thong + ch; DCHch, durian Chani + cholesterol; DKYch, durian Kan Yao + cholesterol. Arrows indicate some differences visible on the gel: DMT differ from others.
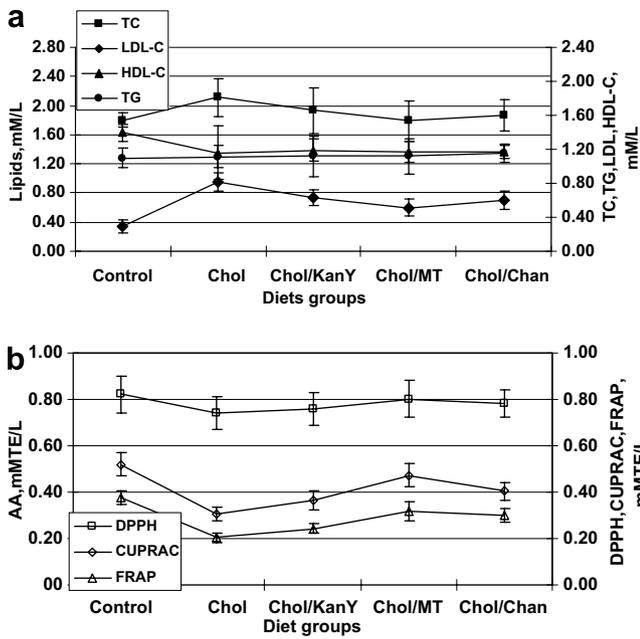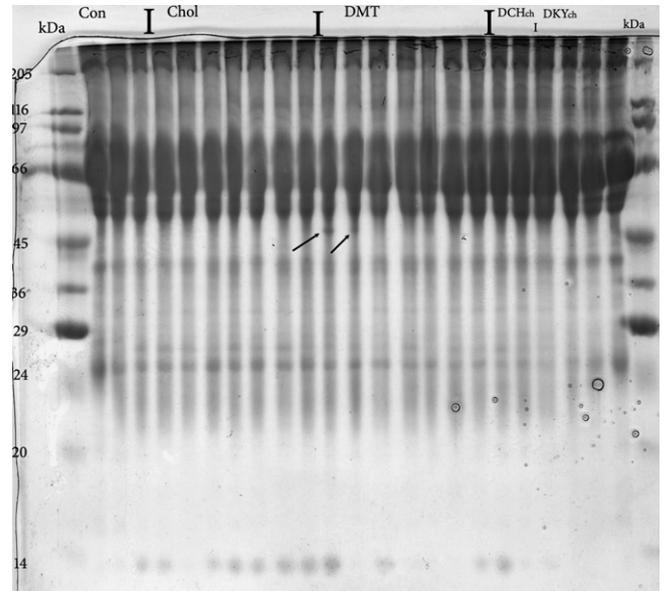
Fig. 5. (a) Changes in the plasma lipid levels after completion of the experiment ($n = 7$); (b) Antioxidant activities in the serum of rats fed different diets. Abbreviations: Diet groups: Chol, Cholesterol; Chol/Kan Y, Kan Yao; Chol/MT, Mon Thong; Chol/Chan, Chani; DPPH, 1, 1-diphenyl-2-picrylhydrazyl; FRAP, Ferric-reducing/antioxidant power; CUPRAC, Cupric reducing antioxidant capacity; total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C) and triglycerides (TG), AA, antioxidant activity.

assays in the rats fed added cholesterol was found (Fig. 5b). However, this decrease was significant only in plasma of rats of the Chol and Chol/Kan Yao diet groups. The

decrease in the plasma antioxidant capacity was predictable, however, it must be underlined that the decrease in the antioxidant activity in the groups of rats fed diets
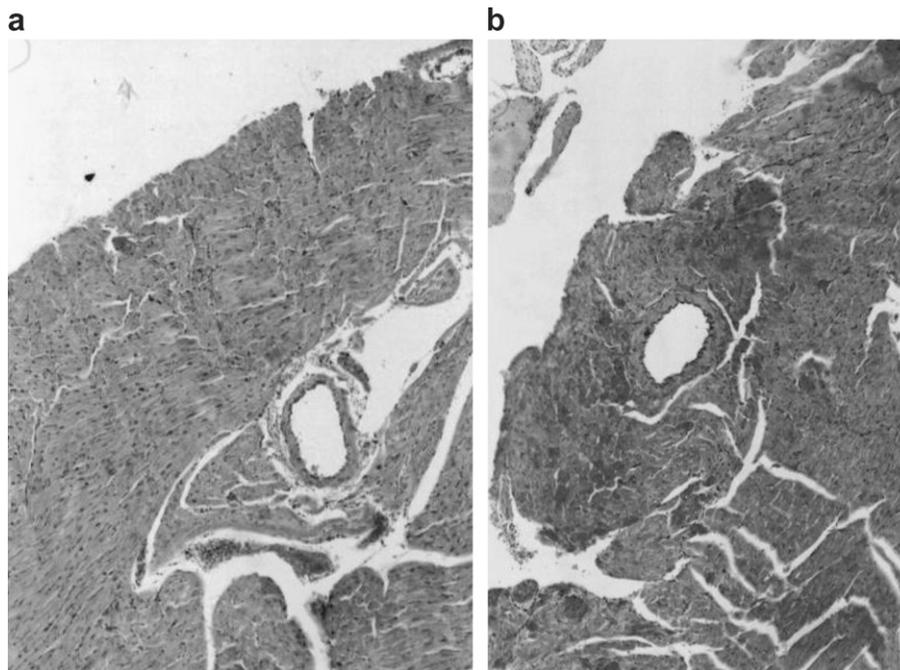

Fig. 7. The histopathological examination of coronary arteries. A, Heart muscle with the cross-section of the coronary artery seen at the center of the photograph; wide arterial lumen and unaltered artery wall. B, Heart muscle with the cross-section of the coronary artery seen in the middle of the photograph. The artery wall exhibits no atherosclerotic lesions.
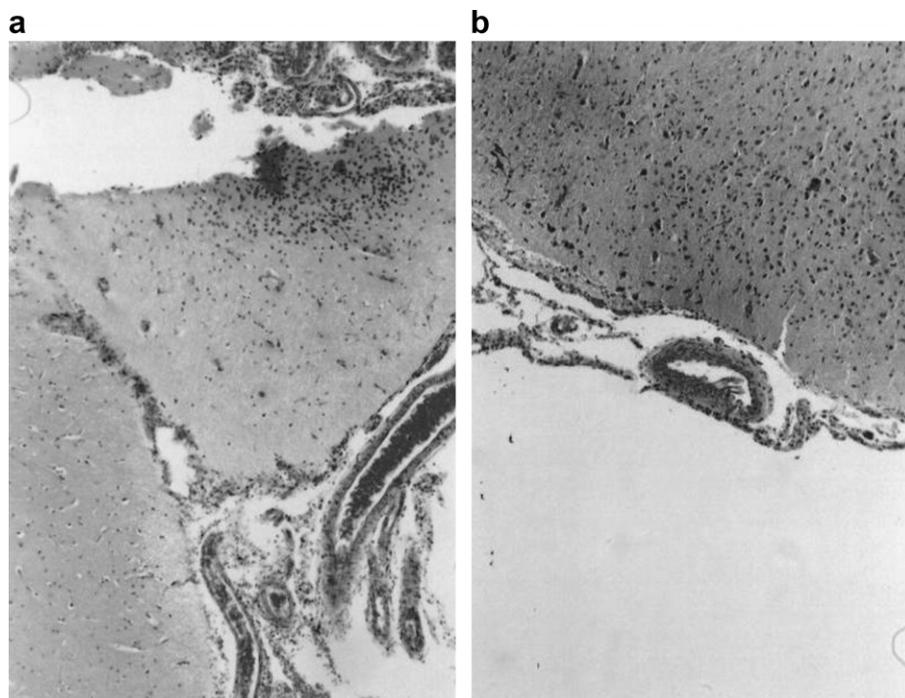
Fig. 8. The histopathological examination of brain arteries. (a) Normal image of the brain tissue with the longitudinal sections of the basal arteries seen right side down. No atherosclerotic changes in the arterial walls. (b) Normal image of the brain tissue with the longitudinal sections of the basal arteries seen right side down. No atherosclerotic changes in the arterial walls.

supplemented with Mon Thong and Chani durian samples (Chol/Mon Thong and Chol/Chani) was significantly less ($P < 0.05$ in both cases) than in Chol and Chol/Kan Yao groups. Therefore, supplementation of diets with Mon Thong and Chani durian cultivars hindered the decrease in the plasma antioxidant activity.

Electrophoretic bands of fibrinogen fraction show a difference between the durian diets. Total rats plasma and the methanol-precipitated fibrinogen fraction during electrophoretic separation detected higher intensity of proteins in the region of 47 kDa (Fig. 6, two arrows). It is especially noticeable for cholesterol diet to have thicker 14 kDa proteins than diets supplemented with Durian fruits.

No lesions were found in the examined tissue of heart and brains (Figs. 7 and 8a–c).

4. Discussion

Many researchers insist on inclusion of fruits and vegetables in disease preventive diets, including atherosclerosis (Prior and Cao, 2000; Kritharides and Stocker, 2002; Kromhout et al., 2002; Luximon-Ramma et al., 2003; Nicolle et al., 2004; Blomhoff, 2005). This positive effect is connected to their bioactive compounds and first of all phenolics (Chau et al., 2004; Kondo et al., 2005).

Tropical fruits have high quantities of these compounds and now some investigators propose to use these fruits in preventive diets (Mahattanatawee et al., 2006; Leontowicz et al., 2007). Some tropical fruits are less known to con-

sumers and among them is durian. The data of the differences between the cultivars practically not exist (Ketsa and Daengkanit, 1999; Voon et al., 2006), therefore, in this investigation three cultivars (Mon Thong, Kan Yao and Chani) of the less investigated durian cultivars were compared.

It was found that the contents of dietary fibers, minerals and trace elements in all three cultivars were high, comparable and similar to other tropical fruits (Mahattanatawee et al., 2006; Leontowicz et al., 2007).

The obtained results are important for the every day diet and were similar to the cited ones, based on the role of dietary fibers and minerals in prevention of some diseases. Potassium had the highest content among the minerals and Fe – among the trace elements (Martinez-Gonzalez et al., 2002; Milton, 2003; Lairon et al., 2005; Leterme et al., 2006; Mahattanatawee et al., 2006; Leontowicz et al., 2007).

The mentioned above minerals participate in the metabolic processes, and influence the prevention of oxidation of lipid fractions.

Total polyphenols and flavonoids were higher in Mon Thong cultivar, and the content of total polyphenols was higher than of flavonoids. The same ratio between these two bioactive compounds (Mahattanatawee et al., 2006; Leontowicz et al., 2007) was found in Snake fruit (*Salacca edulis Reinw*) and Mangosteen (*Garcinia mangostana*). Two each other complemented assays showed that the antioxidant capacity of Mon Thong cultivar was significantly higher than of other two studied cultivars ($P < 0.05$ in both cases). The application of different scavenging methods and

obtained relatively similar results are in agreement with others (Ou et al., 2002). The results *in vitro* correspond with the data of others (Kondo et al., 2005), where the antioxidant activities of guava, mango, banana, rose apple and papaya were analyzed by the same DPPH scavenging method and concluded that the activity in fruit are generally linked with total phenolics. Our results *in vitro* can be compared with other tropical fruits (Mahattanatawee et al., 2006), where the fruits were compared by cultivar and ripening stage and the antioxidant activity depended on these two factors. In our case the three cultivars were in the same ripening stage, therefore the antioxidant activity depended on the type of the cultivar.

A very good correlation was observed between the antioxidant activities determined by DPPH and the total polyphenols. This exactly correspond with the other reports where the antioxidant activity showed high correlations with levels of total polyphenols ($r = 0.96$) where red guava and carambola exhibited the highest activity.

Guava as well as the Mon Thong durian was found to be rich in hydrolysable polyphenols (Mahattanatawee et al., 2006). The correlation coefficient between the polyphenols and antioxidant activity was higher than with the fibers, and this was shown in our previous studies (Gorinstein et al., 2005; Leontowicz et al., 2007). These results were in accordance with others that dietary fiber intake is inversely correlated with several cardiovascular disease risk factors in both sexes, which supports its protective role against cardiovascular disease and recommendations for its increased consumption (Lairon et al., 2005).

Based on the discussed data *in vitro* the results *in vivo* can be supported by the decrease of the lipid levels of cholesterol and the decrease of the plasma antioxidant capacity: Mon Thong has the highest content of bioactive compounds and the highest antioxidant capacity. These results are supported by other reports that if the fruit contains high amount of bioactive compounds than its activity in the biological process is more effective (Paganga et al., 1999). The cholesterol-rich diets have different effects on lipid peroxidation, cholesterol oxides, and antioxidant enzymes in rats and rabbits (Mahfouz and Kummerow, 2000). However, the decrease in groups of rats fed durian cultivars supplemented diets, especially with Mon Thong was significantly less that in Chol group. These results are supported by recently reported data (Suksomtip et al., 2004), where the lipid entrapment property *in vitro* of polysaccharide gel (PG) from fruit-hulls of durian (*Durio zibethinus* L.) was compared with glucomannan (GM), a well studied soluble fiber which showed property of lipid lowering effect by several studies. PG from durian decreased released lipid by a viscosity-mediated interference of lipid releasing outside membrane. PG may have potential use as a supplement for treatment of hyperlipidemic patients. The results obtained in this study can be compared with the use of carrots as a supplement to the diet of animals (Nicolle et al., 2004). In the reviewed study mice were fed either control diets (without or with 0.25%

cholesterol added) or lyophilized carrot enriched diets (20% wt/wt without or with 0.25% cholesterol added) for 4 weeks. This protocol of feeding is different from the one used in our study because we used 1% of cholesterol and 5% of durian. The period of feeding was the same.

Feeding the carrot (Nicolle et al., 2004) diet resulted in a decrease of cholesterol (-41%) and triglycerides (-49%) in plasma and in the liver (-41% and -39%, respectively) in animals fed cholesterol-supplemented diets. These numbers were similar to our results, as well as that the carrot diet increased antioxidant status in cholesterol-fed mice as related by the 16% higher FRAP values. The cited (Nicolle et al., 2004) and our studies show that carrot and durian ingestions decrease lipemia and improve antioxidant status in animals. Such results suggest that fruit intake may exert a protective impact against CAD linked to atherosclerosis. It is likely that these effects could be due to the synergistic effect of fiber and associated antioxidants. Similar to our study (Chau et al., 2004) was done on water-insoluble fiber-rich fraction of carambola and such diet decreased blood serum concentration of serum total cholesterol and liver cholesterol in male Golden Syrian hamsters. The carambola and durian may be a promising cholesterol-lowering ingredient in human diets and fiber-rich functional foods.

The registered results of our investigation concerning the feed intake and body gains are different from the results of others (Anilakumar et al., 2003). However, these authors reported higher feed intake and body gains in rats fed diets supplemented with 10 or 20% of mango fruit bar during 12 weeks of their experiment. In our study the diets were supplemented only with 5% of durian. It was found that due to high nitrogen intake and relatively low nitrogen excretion in feces and urine in the Chol/Mon Thong and to a less degree in Chol/Chani group their nitrogen retention remains significantly higher than in other groups. There are no reports exactly on the nitrogen intake in Durian cultivars, but the aril of Mon Thong durian was firmer and contained less water-soluble pectin and pectinesterase activity than that of Chane (Chanee) durian, while their polygalacturonase activities were comparable during ripening (Gorinstein et al., 2005).

Protein profile of plasma samples showed that in fibrinogen fraction only in Mon Thong cultivars were detected protein bands at 47 kDa. The main patterns were located in the range of 50–116 kDa, showing that the amount of fibrinogen has decreased during the feeding with this diet. These findings indicate that durian consumption leads to decrease in the production of fibrinogen and its stability, which reduces the potential risk exerted by this protein. Therefore from the health point these results are positive. Our data corresponded with others who found improvement of rat blood fluidity and inhibitory effects on the thrombin-induced conversion of fibrinogen to fibrin using the Japanese apricot (Kubo et al., 2005). The histology of heart and brains in our study remained unaffected. In any of the animal groups, no endothelial lesions,

cholesterol deposits, fibrosis or calcification of the intimal and medial layer of the main coronary arteries or their branches were revealed. The microscopic image of the tunica intima and tunica media does not differ between all investigated groups. In the microscopic appearance of brain tissue, there were no endothelial lesions, cholesterol deposits, fibrosis or calcification seen in the intimal and medial layer of the brain arteries. There were found no histopathological changes in the basal cerebral arteries both in the control and the experimental groups. It could be explained by relatively low content of cholesterol in the diets (1%) and the short duration of the experiment (30 days). Also other investigators have shown that only prolonged cholesterol feeding for three and six months induces changes in the histoarchitecture of heart and brains, in the form of fatty streaks and atheromatous plaques followed by fibrous plaques. Our results can be compared with other reports (Tokuno et al., 2002), where too investigate if spontaneous ischemic events in mice with severe multi-organ atherosclerosis could adapt to ischemia, mice were fed an atherogenic diet for 7–9 months. Signs of spontaneous ischemia occurred. One to two days later, hearts were excised, Langendorff-perfused with induced global ischemia, and compared with mice without signs of disease. *In vivo* heart or brain infarctions were verified by heart histology. The finding of the reviewed paper suggests that spontaneous ischemic events in the brain and heart adapt the heart to ischemia. The administered high cholesterol diets elicited in the adopted time no atherosclerotic changes in rat vessels. Under the applied experimental conditions, the rats appeared to be resistant to diet-induced atherosclerosis.

5. Conclusions

All three studied durian cultivars contain high quantities of bioactive compounds, possess high antioxidant capacity and nutritional value, but these indices are significantly higher in Mon Thong. Diets supplemented with durian cultivars improved the plasma lipid levels and plasma antioxidant activity in rats fed cholesterol, therefore durian cultivars especially Mon Thong could be considered as a proper supplement to the disease preventing diets.

Acknowledgment

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