Comparison of the effects of three different Baccaurea angulata *whole fruit juice doses on plasma, aorta and liver MDA levels, antioxidant enzymes and total antioxidant capacity*

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ORIGINAL CONTRIBUTION

Comparison of the effects of three different *Baccaurea angulata* whole fruit juice doses on plasma, aorta and liver MDA levels, antioxidant enzymes and total antioxidant capacity

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Abstract
Purpose *Baccaurea angulata* (common names: belimbing dayak or belimbing hutan) is a Malaysian underutilized fruit. The preliminary work on *B. angulata* fruit juice showed that it possesses antioxidant properties. Therefore, further work is needed to confirm the efficacy and proper dosage of *B. angulata* as a potential natural antioxidant. The present study was thus carried out to compare the effects of three different *B. angulata* whole fruit (WF) juice doses administered at nutritional doses of 0.50, 1.00 and 1.50 ml/kg/day on plasma, aorta and liver malondialdehyde (MDA) levels, antioxidant enzymes (superoxide dismutase, glutathione peroxidase and catalase) as well as total antioxidant capacity in rabbits fed high-cholesterol diet.
Methods Thirty-five male rabbits of New Zealand strain were randomly assigned to seven groups. For 12 weeks, group CH was fed 1% cholesterol diet only; group C1 was fed 1% cholesterol diet and 0.50 ml/kg/day *B. angulata* WF juice; group C2 was fed 1% cholesterol diet and 1.00 ml/kg/day *B. angulata* WF juice; group C3 was fed 1% cholesterol diet and 1.50 ml/kg/day *B. angulata* WF juice; group N was fed standard pellet only; group N1 was fed standard pellet and 0.50 ml/kg/day *B. angulata* WF juice; and group N2 was fed standard pellet and 1.00 ml/kg/day *B. angulata* WF juice.
Results The three doses reduced the formation of MDA and enhanced the expression of endogenous antioxidant enzymes. The highest dose used (1.50 ml/kg/day) was, however, seen as the most potent.
Conclusion Higher doses of *B. angulata* juice exerted better antioxidant activity.

Keywords Antioxidant enzymes · *Baccaurea angulata* · Doses · Malondialdehyde · Underutilized fruit

Introduction

Given the significant lifelong impact of chronic diseases on health and health care costs, effective and affordable interventions for their prevention and control are urgently required. It has never been more important to find real-world solutions to these chronic diseases, especially cardiovascular diseases (CVDs), diabetes and cancers. Therefore, targeting key risk factors for chronic diseases from a variety of angles is an important area for current research. One such approach involves the use of food and food products to combat the common molecular mechanisms that underlie the pathogeneses of cardiovascular and metabolic disorders.

In recent years, increasing evidence suggests that reactive oxygen species (ROS) play a significant contributory role in the development of many chronic diseases such as CVDs and cancers [1]. Oxidative stress occurs when there is a disturbance in the balance between the systemic

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manifestation of ROS and antioxidant defenses; a process that results in altered cellular function, and can cause serious damage to cell structures and tissues [2]. Regulating physiological levels of ROS has become somewhat a potential target for therapeutic intervention for chronic diseases. A popular approach to neutralize free radicals with antioxidants involves using plant compounds [3, 4].

Plant polyphenols have drawn increasing attention due to their potent antioxidant properties [5, 6]. A wealth of evidence has indicated a protective role for a diet rich in fruits in combating free radicals [7, 8]. However, even after years of research, our knowledge still appears too limited regarding which foods have biological activity and how much of a particular food needs to be eaten before a biological effect can be observed.

Preliminary studies on *Baccaurea angulata* fruit juice demonstrated remarkable antioxidant properties of the whole fruit (WF) [9]. Thus, further work is needed to confirm the efficacy and proper dosage of *B. angulata* WF as a potential natural antioxidant. Hence, in the present study, the effects of three different *B. angulata* WF juice doses administered at nutritional doses of 0.50, 1.00 and 1.50 ml/kg/day on plasma, aorta and liver malondialdehyde (MDA) levels, antioxidant enzymes [superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT)] as well as on total antioxidant capacity (TAC) in rabbits fed high-cholesterol diet were compared. It was hypothesized that increasing dose of *B. angulata* WF juice will exert better antioxidant activity.

Materials and methods

Assay kits

Thiobarbituric acid (TBA), reactive substances (TBARS) and TAC assay kits were purchased from Cell Biolabs, Inc. (San Diego, CA 92126, USA). SOD, GPx and CAT assay kits were products of Abnova (Taipei City, Taiwan).

Fruit juice preparation

Every day, healthy whole fruits (obtained from Bau, Sarawak, Malaysia) were washed thoroughly and juiced using an electric fruit juicer just before consumption.

Preparation of cholesterol diet

To prepare hypercholesterolaemic (1% cholesterol) diet, 40 g of pure cholesterol powder (Nacalai-Tesque, Kyoto, Japan) was dissolved in 1.60 l of 99.9% chloroform. The mixture was then mixed with 3.96 kg of standard rabbit pellets under a fume hood. The prepared high-cholesterol diet was oven-dried for 2 days at 40 °C for complete removal of chloroform.

Animals and diets

The animal handling procedure and experimental protocol (Fig. 1) in this study were approved by Animal Care and Use Committee of the Faculty of Medicine, International Islamic University Malaysia (IIUM) Kuantan Campus, Pahang (ID NO. IREC 05; Meeting No. 4/2012) and conformed to the guidelines of the Malaysian Code of Practice for the Care and Use of Animal for Scientific Purposes. A total of 35 healthy adult male rabbits of New Zealand white strains varying in weight from 2.45 ± 0.23 to 2.81 ± 0.27 kg were used in this experiment and were purchased from a certified breeder (A Sapphire Enterprise, Seri Kembangan, Selangor, Malaysia). The animals were randomly housed in an individual stainless-steel cage with free access to food and water in standard constant conditions of lighting, temperature and humidity (20 ± 1 °C, 55 ± 5% humidity with 12-h light/dark cycle) according to approved standards for laboratory animal care. The animals were housed in the animal research laboratory, ground floor, Faculty of Allied Health Science, IIUM. Two weeks of an acclimatization period (all rabbits consumed food and tap water ad libitum) were allowed before initiation of the experimental protocol. Following acclimatization, the rabbits were randomly allocated to seven experimental groups with five rabbits in each group. Each rabbit was fed 120 g/day for 12 weeks. Group CH (hypercholesterolaemic control) was fed 1% cholesterol diet only; group C1 (hypercholesterolaemic low dose) was fed 1% cholesterol diet and 0.50 ml/kg/day *B. angulata* WF juice; group C2 (hypercholesterolaemic medium dose) was fed 1% cholesterol diet and 1.00 ml/kg/day *B. angulata* WF juice; group C3 (hypercholesterolaemic high dose) was fed 1% cholesterol diet and 1.50 ml/kg/day *B. angulata* WF juice; group N (normocholesterolaemic control) was fed standard pellet only; group N1 (normocholesterolaemic low dose) was fed standard pellet and 0.50 ml/kg/day *B. angulata* WF juice; and group N2 (normocholesterolaemic medium dose) was fed standard pellet and 1.00 ml/kg/day *B. angulata* WF juice (Fig. 1). Juice was administered orally using a clean syringe and the doses were selected based on the previous research [10] on the sub-chronic toxicological evaluation of *B. angulata* fruit juice in rats, administered orally for 13 weeks. It was reported that the no-observable adverse effect level of *B. angulata* fruit juice was 1.20 g/kg (1.20 ml/kg). The use of these three dose levels (0.50, 1.00 and 1.50 ml/kg) for rabbits has also been reported for *Fagonia cretica* fruit [11], fish oil (omega-3) [12] and curcumin [13].

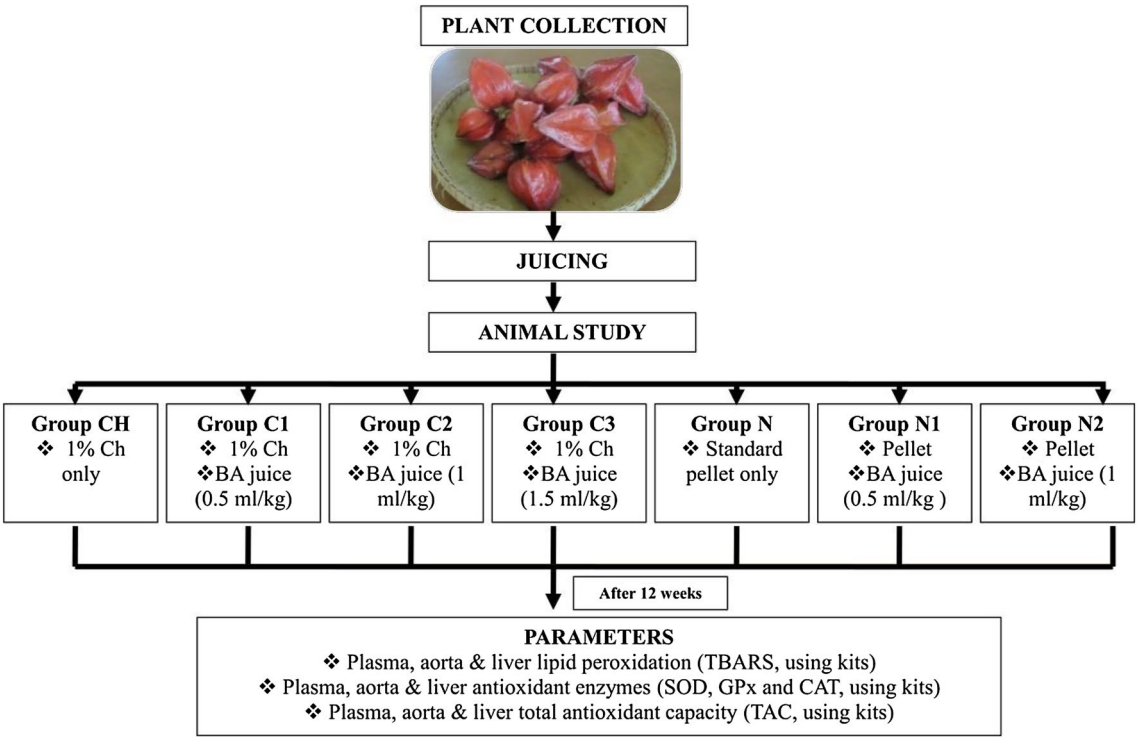

Fig. 1 Experimental design of the study on the comparison of the effects of three different *B. angulata* WF juice doses on plasma, aorta and liver MDA levels, antioxidant enzymes and total antioxidant capacity (TAC). *Ch* cholesterol diet, *TBARS* thiobarbituric acid reactive substances (MDA quantitation), *SOD* superoxide dismutase, *GPx* glutathione peroxidase, *CAT* catalase

Food intake was measured daily, and the body weight of rabbits was measured weekly. Juice doses were always adjusted based on the weekly weight of each animal. The animals received proper care in compliance with the animal care and use.

Blood, aorta and liver collection

Rabbits were deprived of food overnight prior to blood collection from the marginal ear vein into heparinised tubes at the beginning and the end of the experiment (after the 12-week feeding period) [14–16]. Blood samples were stored on ice until being centrifuged at 1800*g* and 4 °C for 15 min. Plasma was aliquoted and stored at −80 °C until use. All rabbits were then killed; their abdomens and chests opened; heart, aorta, liver, lung and kidney collected; washed thoroughly with cold phosphate-buffered saline (PBS) and weighed. Segments between the first and the second intercostal arteries were cut from the aorta. From each liver, a small segment was cut from the right lobe; tissues were stored in foil pouches and immediately snap-frozen in liquid nitrogen. They were later stored at −80 °C until use.

Aorta and liver homogenates preparation

Aorta and liver were defrosted before homogenization in radioimmunoprecipitation assay (RIPA) buffer containing a protease inhibitor [17]. The solution was centrifuged at 10,000*g* for 5 min at 4 °C to collect the supernatant (homogenates). The supernatant was stored at −80 °C and assayed for protein concentration using Coomassie (Bradford) protein assay [18]. Bovine serum albumin (Nacalai-Tesque, Japan) was used as a standard. Samples were expressed as units per milligram of tissue homogenate protein.

Lipid peroxidation assay

To measure the levels of MDA in plasma (week 0 and week 12), aorta and liver homogenates, TBARS assay (Cell Biolabs, Inc., catalog no. STA-330) was employed. In this kit, the unknown MDA containing samples forms a 1:2 adduct with TBA. The MDA-TBA adduct formed was measured spectrophotometrically at 532 nm. The analysis was done according to the manufacturer's instructions.

Antioxidant enzyme activities

The levels of SOD, GPx and CAT activities as well as TAC in plasma (week 0 and week 12), aorta and liver homogenates were estimated spectrophotometrically using commercial assay kits (Abnova; Taipei City, Taiwan and Cell Biolabs, Inc. San Diego, CA 92126, USA). The analyses were done according to the manufacturer's instructions.

Statistical analysis

Data were expressed as means ± standard deviations (SD). The data were analyzed by ANOVA using Statistical Package for Social Sciences (SPSS) software (version 20.0, IBM) and the differences between the means were compared by Duncan's multiple-range test. Two-way ANOVA was used to study the effects of cholesterol, dose and dose–cholesterol interaction while repeated measures ANOVA was used to study the effects of time, treatment and time–treatment interaction. Statistical significance was considered at $p < 0.05$.

Results

Effects of *B. angulata* WF juice doses on body weight gain

Table 1 shows the weight gain of the rabbits. In the present study, no significant difference ($p > 0.05$) was observed in the average weight gain between all groups at the end of the feeding trial. Cholesterol and *B. angulata* WF juice doses significantly influenced ($p = 0.013$ and $p = 0.001$, respectively) body weight gain.

Effects of *B. angulata* WF juice doses on daily feed intake

Table 1 shows that food intake was significantly affected by both cholesterol ($p = 0.009$) and *B. angulata* WF juice doses ($p = 0.005$). The average daily feed intakes of diets with or without cholesterol in this study were between 0.08 and 0.11 kg.

Toxic effects of *B. angulata* WF juice

No apparent toxic effects of *B. angulata* WF juice, including effects on growth and food consumption, were observed. All rabbits maintained good health during the entire course of the study and survived till the end of the study.

Effects of *B. angulata* WF juice doses on relative weights of organs

The results of relative weights of organs are shown in Table 1. Group C2 had the highest relative heart's weight and relative liver's weight, while the lowest relative heart's weight and relative liver's weight were observed in the group N. The CH group had the highest relative lung's weight, while the lowest relative lung's weight was observed in the N group. The C3 group had the highest relative kidney's weight; the N group, however, had the lowest relative kidney's weight. The C3 group showed slightly lower relative liver weight than other cholesterol-fed rabbits. The relative lung and kidney weights did not significantly differ ($p > 0.05$) between all groups except for relative lung weight in groups N and CH ($p < 0.05$).

Effects of *B. angulata* WF juice doses on plasma malondialdehyde

The plasma MDA levels of the various groups are given in Table 2. The high-cholesterol diet caused a marked increase in plasma MDA levels in CH, C1, C2 and C3 groups after 12 weeks of treatment. However, in groups C1, C2 and C3, plasma MDA concentrations were significantly lower ($p < 0.05$) when compared to the CH group. Group CH had the highest plasma MDA concentration. On the contrary, the 1.50 ml/kg/day *B. angulata* WF-treated group (C3) had the lowest plasma MDA concentration. Significant time–treatment ($p < 0.001$) (Table 2) and cholesterol–dose ($p = 0.008$) (Table 3) interactions were found.

Effects of *B. angulata* WF juice doses on plasma superoxide dismutase activity

Table 2 presents the plasma SOD activities of the various groups. The mean baseline SOD activities of all the groups were not significantly different ($p > 0.05$) from each other. Significantly higher ($p < 0.05$) mean plasma SOD activities were observed in C1, C2 and C3 groups when compared with CH group after 12 weeks of the experimental study. Groups N, N1 and N2 exhibited increases in their plasma SOD activities. Group N2 had a higher percentage increase (9.34%) in plasma SOD activity than groups N1 and N, while group N1 had a higher percentage increase (3.68%) in plasma SOD activity than group N (2.90%). Significant time–treatment interaction ($p < 0.001$) (Table 2) was found and cholesterol–dose interaction was of borderline statistical significance ($p = 0.064$) (Table 3). In our study, when considering the percentage changes, 1.50 ml/kg/day *B. angulata* WF juice showed effect higher than those of 0.50

Table 1 Weight gain, daily feed intake, and relative weights of organs in normocholesterolaemic and hypercholesterolaemic rabbits

Parameter	Control	Low dose	Medium dose	High dose[d]	Statistical significance (two-way ANOVA)		
					Cholesterol effect	Dose effect	Cholesterol × dose
Weight gain (kg)							
Hypercholesterolaemic	0.17 ± 0.09^{a}	0.09 ± 0.10^{a}	0.10 ± 0.36^{a}	0.06 ± 0.29^{a}	$p = 0.013$	$p = 0.001$	$p = 0.057$
Normocholesterolaemic	0.22 ± 0.08^{a}	0.09 ± 0.13^{a}	0.15 ± 0.23^{a}	NA			
Daily feed intake (kg)							
Hypercholesterolaemic	$0.10 \pm 0.01^{a,b}$	0.08 ± 0.01^{a}	$0.09 \pm 0.01^{a,b}$	$0.10 \pm 0.02^{a,b}$	$p = 0.009$	$p = 0.005$	NS
Normocholesterolaemic	0.11 ± 0.01^{b}	$0.10 \pm 0.02^{a,b}$	$0.10 \pm 0.01^{a,b}$	NA			
Relative heart weight (%)							
Hypercholesterolaemic	$0.24 \pm 0.02^{a,b}$	0.26 ± 0.05^{b}	0.28 ± 0.06^{b}	0.26 ± 0.05^{b}	$p < 0.001$	$p = 0.017$	NS
Normocholesterolaemic	0.18 ± 0.01^{a}	0.19 ± 0.03^{a}	$0.21 \pm 0.04^{a,b}$	NA			
Relative liver weight (%)							
Hypercholesterolaemic	$3.35 \pm 0.56^{b,c}$	$3.46 \pm 0.68^{b,c}$	4.09 ± 0.92^{c}	2.87 ± 0.18^{b}	$p < 0.001$	$p = 0.022$	NS
Normocholesterolaemic	1.32 ± 0.23^{a}	1.66 ± 0.34^{a}	1.72 ± 0.15^{a}	NA			
Relative lung weight (%)							
Hypercholesterolaemic	0.42 ± 0.06^{b}	$0.40 \pm 0.06^{a,b}$	$0.39 \pm 0.04^{a,b}$	$0.36 \pm 0.04^{a,b}$	$p = 0.005$	NS	$p = 0.026$
Normocholesterolaemic	0.33 ± 0.03^{a}	$0.34 \pm 0.03^{a,b}$	$0.38 \pm 0.10^{a,b}$	NA			
Relative kidney weight (%)							
Hypercholesterolaemic	$0.47 \pm 0.06^{a,b}$	0.50 ± 0.03^{b}	0.51 ± 0.03^{b}	0.54 ± 0.15^{b}	$p = 0.001$	$p = 0.014$	NS
Normocholesterolaemic	0.37 ± 0.04^{a}	$0.43 \pm 0.07^{a,b}$	$0.45 \pm 0.04^{a,b}$	NA			

Values are given as means ± standard deviations ($n = 5$)

Hypercholesterolaemic control (CH), 1% cholesterol diet only; hypercholesterolaemic low dose (C1), 1% cholesterol diet and 0.50 ml/kg/day *B. angulata* WF juice; hypercholesterolaemic medium dose (C2), 1% cholesterol diet and 1.00 ml/kg/day *B. angulata* WF juice; hypercholesterolaemic high dose (C3), 1% cholesterol diet and 1.50 ml/kg/day *B. angulata* WF juice; normocholesterolaemic control (N), standard pellet only; normocholesterolaemic low dose (N1), standard pellet and 0.50 ml/kg/day *B. angulata* WF juice; normocholesterolaemic medium dose (N2), standard pellet and 1.00 ml/kg/day *B. angulata* WF juice; NA, not available; NS, not significant

[a, b, c] Values not sharing a common superscript letter differ significantly at $p < 0.05$ for each parameter (post hoc analysis)

[d] Not included in the two-way ANOVA analysis

Table 2 Baseline (wk 0) and final (wk 12) plasma malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), total antioxidant capacity (TAC) and body weight in normocholesterolaemic and hypercholesterolaemic rabbits

Plasma	CH	C1	C2	C3	N	N1	N2	Repeated measures ANOVA		
								Time effect	Treatment effect	Time × treatment
MDA wk0 (μM)	4.25 ± 1.88^{a}	3.81 ± 1.09^{a}	3.79 ± 2.33^{a}	5.68 ± 5.30^{a}	3.55 ± 0.62^{a}	4.74 ± 2.00^{a}	3.95 ± 0.67^{a}	$p < 0.001$	$p < 0.001$	$p < 0.001$
MDA wk12 (μM)	133 ± 33.0^{a}	79.7 ± 21.4^{b}	29.3 ± 4.21^{c}	23.1 ± 7.09^{c}	3.92 ± 1.02^{d}	3.69 ± 1.07^{d}	2.91 ± 0.78^{d}			
SOD wk0 (%)	71.5 ± 8.27^{a}	79.9 ± 11.4^{a}	72.9 ± 16.1^{a}	62.2 ± 23.2^{a}	77.8 ± 12.6^{a}	74.2 ± 7.41^{a}	73.2 ± 8.61^{a}	$p = 0.019$	$p < 0.001$	$p < 0.001$
SOD wk12 (%)	34.9 ± 3.42^{a}	71.0 ± 8.80^{b}	59.3 ± 14.7^{b}	69.1 ± 16.7^{b}	80.1 ± 14.6^{b}	76.9 ± 12.7^{b}	80.1 ± 13.7^{b}			
GPx wk0 (U/L)	144 ± 14.4^{a}	120 ± 7.55^{b}	120 ± 16.0^{b}	150 ± 14.4^{a}	$127 \pm 17.0^{a,b}$	129 ± 22.7^{b}	$131 \pm 6.11^{a,b}$	$p < 0.001$	$p < 0.001$	$p < 0.001$
GPx wk12 (U/L)	83.0 ± 17.9^{a}	161 ± 8.89^{b}	215 ± 51.0^{c}	295 ± 14.1^{c}	$122 \pm 17.5^{a,b}$	265 ± 54.1^{c}	258 ± 36.2^{c}			
CAT wk0 (U/L)	4.49 ± 0.58^{a}	4.32 ± 0.29^{a}	4.23 ± 0.15^{a}	4.72 ± 0.19^{a}	3.32 ± 1.69^{a}	3.99 ± 0.77^{a}	3.97 ± 0.87^{a}	$p < 0.001$	$p < 0.001$	$p < 0.001$
CAT wk12 (U/L)	1.50 ± 0.18^{a}	$2.47 \pm 1.43^{a,b}$	3.49 ± 0.67^{b}	4.16 ± 0.84^{b}	$3.33 \pm 1.70^{a,b}$	4.30 ± 0.98^{b}	3.79 ± 1.48^{b}			
TAC wk0 (mM)	245 ± 11.8^{a}	241 ± 44.7^{a}	182 ± 58.3^{a}	225 ± 97.4^{a}	203 ± 30.0^{a}	185 ± 25.2^{a}	161 ± 29.0^{a}	$p < 0.001$	NS	$p = 0.006$
TAC wk12 (mM)	225 ± 8.75^{a}	246 ± 92.4^{a}	236 ± 54.8^{a}	300 ± 63.3^{a}	207 ± 24.1^{a}	251 ± 70.6^{a}	295 ± 50.4^{a}			
Weight wk0 (kg)	2.64 ± 0.21^{a}	2.45 ± 0.23^{a}	2.50 ± 0.50^{a}	2.70 ± 0.34^{a}	2.81 ± 0.27^{a}	2.56 ± 0.22^{a}	2.39 ± 0.22^{a}	$p < 0.001$	$p < 0.001$	NS
Weight wk12 (kg)	$2.81 \pm 0.24^{a,b}$	2.55 ± 0.17^{a}	2.59 ± 0.19^{a}	$2.75 \pm 0.32^{a,b}$	3.03 ± 0.34^{b}	$2.65 \pm 0.30^{a,b}$	2.54 ± 0.24^{a}			

Values are given as means ± standard deviations ($n = 5$)

CH, 1% cholesterol diet only; C1, 1% cholesterol diet and 0.50 ml/kg/day *B. angulata* WF juice; C2, 1% cholesterol diet and 1.00 ml/kg/day *B. angulata* WF juice; C3, 1% cholesterol diet and 1.50 ml/kg/day *B. angulata* WF juice; N, standard pellet only; N1, standard pellet and 0.50 ml/kg/day *B. angulata* WF juice; N2, standard pellet and 1.00 ml/kg/day *B. angulata* WF juice; NS, not significant

[a, b, c, d] Values not sharing a common superscript letter within the same row differ significantly at $p < 0.05$ (post hoc analysis)

Table 3 The effects of cholesterol, dose and dose–cholesterol interaction on plasma malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and total antioxidant capacity (TAC)

	Control	Low dose	Medium dose	Statistical significance (two-way ANOVA)		
				Cholesterol effect	Dose effect	Cholesterol × dose
Plasma MDA (μM)						
Hypercholesterolaemic	133 ± 33.0	79.7 ± 21.4	29.3 ± 4.21	$p < 0.001$	$p = 0.009$	$p = 0.008$
Normocholesterolaemic	3.92 ± 1.02	3.69 ± 1.07	2.91 ± 0.78			
Plasma SOD (%)						
Hypercholesterolaemic	34.9 ± 3.42	71.0 ± 8.80	59.3 ± 14.7	$p = 0.009$	$p = 0.001$	$p = 0.064$
Normocholesterolaemic	80.1 ± 14.6	76.9 ± 12.7	80.1 ± 13.7			
Plasma GPx (U/L)						
Hypercholesterolaemic	83.0 ± 17.9	161 ± 8.89	215 ± 51.0	$p = 0.001$	$p = 0.002$	$p = 0.015$
Normocholesterolaemic	122 ± 17.5	265 ± 54.1	258 ± 36.2			
Plasma CAT (U/L)						
Hypercholesterolaemic	1.50 ± 0.18	2.47 ± 1.43	3.49 ± 0.67	$p = 0.036$	$p = 0.031$	NS
Normocholesterolaemic	3.33 ± 1.70	4.30 ± 0.98	3.79 ± 1.48			
Plasma TAC (mM)						
Hypercholesterolaemic	225 ± 8.75	246 ± 92.4	236 ± 54.8	NS	$p = 0.083$	NS
Normocholesterolaemic	206 ± 24.1	251 ± 70.6	295 ± 50.4			

Values are given as means ± standard deviations ($n = 5$)

Hypercholesterolaemic control (CH), 1% cholesterol diet only; hypercholesterolaemic low dose (C1), 1% cholesterol diet and 0.50 ml/kg/day *B. angulata* WF juice; hypercholesterolaemic medium dose (C2), 1% cholesterol diet and 1.00 ml/kg/day *B. angulata* WF juice; normocholesterolaemic control (N), standard pellet only; normocholesterolaemic low dose (N1), standard pellet and 0.50 ml/kg/day *B. angulata* WF juice; normocholesterolaemic medium dose (N2), standard pellet and 1.00 ml/kg/day *B. angulata* WF juice; NS, not significant

and 1.00 ml/kg/day. Likewise, this effect was more prominent in rabbits given standard pellet and 1.00 ml/kg/day *B. angulata* WF juice (group N2) than in rabbits of standard pellet and 0.50 ml/kg/day *B. angulata* WF juice (group N1).

Effects of *B. angulata* WF juice doses on plasma glutathione peroxidase activity

Plasma GPx activities of the various groups are shown in Table 2. Variations were observed in the mean baseline GPx activities. After 12 weeks of the experimental study, among the groups CH, C1, C2 and C3, level of plasma GPx activity decreased in group CH, but in groups C1, C2 and C3, a significant increase ($p < 0.05$) in plasma GPx activities was observed with a dose-dependent pattern. Conversely, the administration of *B. angulata* WF juices resulted in about 34, 79 and 96% increases of plasma GPx activities (Table 2) in groups C1, C2 and C3, respectively. Among groups N, N1 and N2, only group N exhibited a decrease in plasma GPx activity after 12 weeks of experiment, whereas groups N1 and N2 had percentage increases of 105 and 96.7%, respectively. Time–treatment (Table 2) and cholesterol–dose interactions (Table 3) were both statistically significant ($p < 0.001$ and $p = 0.015$, respectively).

Effects of *B. angulata* WF juice doses on plasma catalase activity

The CAT activities in plasma of rabbits are given in Table 2. When compared the three dose groups of animals (C1, C2 and C3) with group CH, the effect was significant ($p < 0.05$) at the two higher doses (1.00 and 1.50 ml/kg/day). After 12 weeks of the experimental study, the effect of *B. angulata* WF juice on plasma CAT activities in rabbits fed high-cholesterol diet was dose dependent. A contrasting effect was observed in groups

N1 and N2. Though group N1 had a higher percentage increase than group N, group N2, however, had a percentage decrease of −4.53%. A significant time–treatment interaction ($p < 0.001$) (Table 2) was found.

Effects of *B. angulata* WF juice doses on plasma total antioxidant capacity

The baseline and final TAC levels of the seven groups of rabbits are summarized in Table 2. The CH group had a percentage decrease of −8.20%, while the groups C1, C2 and C3 had percentage increases of 1.89, 30.1 and 33.5%, respectively. A dose-dependent pattern and a comparatively higher percentage increases in TAC levels were observed in groups N1 (36.0%) and N2 (83.2%) compared to group N (2.48%). A significant time–treatment interaction ($p = 0.006$) (Table 2) was found.

Effects of *B. angulata* WF juice doses on aorta levels of MDA, SOD, GPx, CAT and TAC

The aorta levels of MDA, SOD, GPx, CAT and TAC in all the rabbit groups are illustrated in Table 4. After 12 weeks of treatment, the aorta MDA level of group CH rabbits showed a significant increase ($p < 0.05$) compared with that of rabbits fed the standard pellet only (group N). However, a significant decrease ($p < 0.05$) of aorta MDA levels of rabbits fed both high-cholesterol diet and *B. angulata* WF juices (C1, C2 and C3 groups) was observed. The rabbits orally treated with 1.50 ml/kg/day *B. angulata* WF juice (group C3) had lower, though insignificant ($p > 0.05$) aorta MDA level than those of rabbits in C1 and C2 groups.

Although the aorta SOD activity level of rabbits fed high-cholesterol diet only (group CH) was not significantly different ($p > 0.05$) from those of groups C1, C2 and C3, the level in group CH, however, was outstandingly lower than those in the other three groups. Groups C1, C2 and C3 showed no dose-dependent pattern.

As shown in Table 4, *B. angulata* WF-treated rabbits (groups C1, C2 and C3) showed a remarkably significant increase ($p < 0.05$) in the aorta GPx activity levels as compared with the rabbits fed high-cholesterol diet only (group CH). The aorta GPx activity level significantly increased ($p < 0.05$) in a dose-dependent manner by orally administering *B. angulata* WF juices. There was no significant difference ($p > 0.05$) in the aorta GPx activity levels of groups N and N1. Group N2, on the other hand, had a significantly higher ($p < 0.05$) aorta GPx activity level than groups N and N1.

An oral administration of low dose (0.50 ml/kg/day), medium dose (1.00 ml/kg/day) and high dose (1.50 ml/kg/day) of *B. angulata* WF juices did not significantly increase ($p > 0.05$) the aorta CAT activity levels of rabbits compared with those of rabbits fed high-cholesterol diet only (group CH). Likewise, aorta CAT activity levels of rabbits in both N1 and N2 were insignificantly higher ($p > 0.05$) than that of rabbits in N group.

The aorta TAC levels of rabbits in groups C1, C2 and C3 significantly increased ($p < 0.05$) compared with that of rabbits in group CH. The groups N1 and N2 had higher aorta TAC levels than group N. The results, however, were not significantly different ($p > 0.05$). Significant cholesterol–dose interactions on aorta MDA ($p < 0.001$), SOD ($p = 0.044$), GPx ($p = 0.002$) and TAC ($p = 0.001$) were found.

Effects *B. angulata* WF juice doses on hepatic levels of MDA, SOD, GPx, CAT and TAC

Table 4 shows the hepatic levels of MDA, SOD, GPx, CAT and TAC in all the rabbit groups. MDA level was significantly lower ($p < 0.05$) in the liver tissues of rabbits in group C2 compared with that of group CH. Hepatic MDA levels of groups N1 and N2 did not differ significantly ($p > 0.05$) from that of group N.

The activity of hepatic SOD was significantly lower ($p < 0.05$) in group CH compared with other groups, except for group C1 ($p > 0.05$). Groups N1 and N2 had significantly elevated ($p < 0.05$) hepatic SOD activities compared with that of group N.

Groups C1, C2 and C3 had significantly higher ($p < 0.05$) hepatic GPx activities than group CH. No significant difference ($p > 0.05$) was observed in the hepatic GPx activities of groups N, N1 and N2.

The activities of hepatic CAT in groups C1, C2 and C3 were significantly higher ($p < 0.05$) than that of group CH. On the contrary, hepatic CAT activity levels of groups N1 and N2 did not differ significantly ($p > 0.05$) from that of group N.

The TAC values in the liver of rabbits significantly increased ($p < 0.05$) after treatment with 1.50 ml/kg/day *B. angulata* WF juice (group C3) compared to that of group CH. Significant cholesterol–dose interactions on hepatic MDA ($p = 0.016$), SOD ($p = 0.039$), GPx ($p = 0.044$) and CAT ($p = 0.002$) were found.

Discussion

Earlier study has reported the phytochemical constituents and antioxidant activities of various parts (whole fruit, skin and pulp) of *B. angulata* fruit [9, 19]. In this present study, the whole fruit was chosen for the animal study, as it was the part that contained the highest concentrations of

Table 4 Aorta and liver plasma malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and total antioxidant capacity (TAC) in normocholesterolaemic and hypercholesterolaemic rabbits

Parameter	Control	Low dose	Medium dose	High dose[f]	Statistical significance (two-way NOVA)		
					Cholesterol effect	Dose effect	Cholesterol × dose
Aorta MDA (μM/mg protein)							
Hypercholesterolaemic	44.9 ± 2.62[a]	6.42 ± 1.08[b]	9.18 ± 6.24[b]	5.58 ± 1.95[b]	$p < 0.001$	$p < 0.001$	$p < 0.001$
Normocholesterolaemic	4.80 ± 2.35[b]	2.78 ± 0.83[b]	7.61 ± 5.93[b]	NA			
Aorta SOD (%)							
Hypercholesterolaemic	32.9 ± 5.02[a]	45.3 ± 29.6[a]	65.1 ± 20.5[a]	48.3 ± 28.1[a]	$p = 0.007$	NS	$p = 0.044$
Normocholesterolaemic	71.7 ± 20.8[a]	76.4 ± 25.4[a]	61.5 ± 27.7[a]	NA			
Aorta GPx (U/mg protein)							
Hypercholesterolaemic	85.7 ± 4.51[a]	199 ± 10.5[b]	253 ± 25.3[c]	263 ± 40.7[c]	$p = 0.024$	$p < 0.001$	$p = 0.002$
Normocholesterolaemic	124 ± 9.29[a]	128 ± 3.51[a]	249 ± 42.2[b,c]	NA			
Aorta CAT (U/mg protein)							
Hypercholesterolaemic	3.14 ± 1.35[a,b]	2.23 ± 1.07[a]	3.11 ± 0.43[a,b]	3.74 ± 0.42[b,c]	$p = 0.001$	$p = 0.171$	$p = 0.087$
Normocholesterolaemic	3.98 ± 0.49[b,c]	4.23 ± 0.47[b,c]	4.66 ± 0.30[c]	NA			
Aorta TAC (mM/mg protein)							
Hypercholesterolaemic	119 ± 26.2[a]	333 ± 8.67[b,c]	303 ± 61.8[b]	250 ± 58.6[b]	$p < 0.001$	$p < 0.001$	$p = 0.001$
Normocholesterolaemic	336 ± 25.7[b,c]	439 ± 30.9[c]	436 ± 30.1[c]	NA			
Liver MDA (μM/mg protein)							
Hypercholesterolaemic	58.4 ± 19.6[c]	37.4 ± 9.49[b,c]	30.2 ± 9.86[a,b]	41.3 ± 18.6[b,c]	$p = 0.001$	$p = 0.004$	$p = 0.016$
Normocholesterolaemic	13.4 ± 0.94[a]	23.9 ± 3.84[a,b]	10.7 ± 3.51[a]	NA			
Liver SOD (%)							
Hypercholesterolaemic	5.72 ± 2.19[a]	12.0 ± 6.35[a,b]	33.0 ± 4.95[b,c]	43.4 ± 13.1[c,d]	$p < 0.001$	$p < 0.001$	$p = 0.039$
Normocholesterolaemic	32.8 ± 9.73[b,c]	56.4 ± 26.0[d]	86.1 ± 8.02[e]	NA			
Liver GPx (U/mg protein)							
Hypercholesterolaemic	98.7 ± 9.87[a]	124 ± 6.66[b]	132 ± 7.09[b]	158 ± 26.5[c]	$p = 0.006$	$p = 0.002$	$p = 0.044$
Normocholesterolaemic	129 ± 15.1[b]	131 ± 12.5[b]	138 ± 8.74[b,c]	NA			
Liver CAT (U/mg protein)							
Hypercholesterolaemic	1.74 ± 0.26[a]	3.64 ± 0.66[b]	3.62 ± 1.07[b]	4.46 ± 0.08[b]	$p = 0.055$	$p = 0.002$	$p = 0.002$
Normocholesterolaemic	3.32 ± 0.51[b]	3.19 ± 1.52[b]	3.85 ± 0.16[b]	NA			
Liver TAC (mM/mg protein)							
Hypercholesterolaemic	167 ± 71.4[a]	241 ± 54.3[a,b]	254 ± 30.2[a,b]	308 ± 39.3[b]	$p < 0.001$	$p < 0.137$	NS
Normocholesterolaemic	298 ± 30.3[b]	345 ± 48.3[b]	353 ± 46.7[b]	NA			

Values are given as means ± standard deviations ($n = 5$)

Hypercholesterolaemic control (CH), 1% cholesterol diet only; hypercholesterolaemic low dose (C1), 1% cholesterol diet and 0.50 ml/kg/day *B. angulata* WF juice; hypercholesterolaemic medium dose (C2), 1% cholesterol diet and 1.00 ml/kg/day *B. angulata* WF juice; hypercholesterolaemic high dose (C3), 1% cholesterol diet and 1.50 ml/kg/day *B. angulata* WF juice; normocholesterolaemic control (N), standard pellet only; normocholesterolaemic low dose (N1), standard pellet and 0.50 ml/kg/day *B. angulata* WF juice; normocholesterolaemic medium dose (N2), standard pellet and 1.00 ml/kg/day *B. angulata* WF juice; NA, not available; NS, not significant

[a, b, c, d, e] Values not sharing a common superscript letter differ significantly at $p < 0.05$ for each parameter (post hoc analysis)

[f] Not included in the two-way ANOVA analysis

total flavonoids (1.30 g/kg dry weight) and phenolic acids (0.71 g/kg dry weight). Quercetin (0.53 g/kg dry weight), catechin (0.52 g/kg dry weight) and kaempferol (0.18 g/kg dry weight) were the major flavonoids from the five analyzed. Ferulic acid (0.31 g/kg dry weight), sinapic acid (0.15 g/kg dry weight) and vanillic acid (0.09 g/kg dry weight) were the major phenolic acids in *B. angulata* WF, equivalent to 43.4, 22.5 and 13.1% of the total ten phenolic acids' concentration, respectively (authors' unpublished data). The result of the present study is in agreement with the findings of other researchers with regard to weight gain of rabbits fed cholesterol-enriched diet. Chen et al. [20] revealed that there was no difference in body weight gain between the four groups of rabbits fed cholesterol diet. However, Bocanegra et al. [21] reported a decrease in body weight gain due to cholesterol feeding. The result also indicates that *B. angulata* juice is safe. Several animal studies have reported no toxic effects after fruit juice administration [22–24].

Relative weight of organs is an important determinant of hypertrophy. Feeding animals with cholesterol-enriched diet commonly results in an increase in body weight and relative organ's weight with abnormal macroscopic appearance [25] as well as an increase in the content of cholesterol in rat's heart [26]. In the present study, high-cholesterol diets literally clog up the liver and thus cause changes in the adipocytes (hypertrophy). This deleterious effect of high-cholesterol diet on liver can have far-reaching consequences for health [27]. Several studies have reported the increased liver length, width and weight in high-cholesterol fed animals [28–31]. The slightly lower relative liver weight of the C3 group compared to other cholesterol-fed rabbits could be possibly a result of increased excretion or metabolism of cholesterol. This, therefore, indicates that when enough *B. angulata* WF serving is taken, it might exert liver cleansing effect.

The results suggest that *B. angulata* WF significantly reduced ($p < 0.05$) plasma MDA in a dose-dependent manner. These are consistent with the work of Oboh et al. [32], who reported the inhibitory effect of phenolic-rich extracts from *Allium sativum* (garlic) on angiotensin-converting enzyme (ACE) activity and cisplatin-induced lipid peroxidation in rat kidney in vitro. Extensive studies on the importance of the well-known and underutilized fruits in preventing lipid peroxidation are gaining much attention. Phenolic-rich *Citrus maxima* inhibited MDA production in cultured rats' pancreas [33]. Likewise, the polyphenol- and carotenoid-rich *Mangifera indica* L. peel extract showed protection against hydrogen peroxide-induced oxidative damage in rat erythrocytes [34]. Citrus fruit juices scavenged 1,1-diphenyl-2-picrylhydrazyl and hydroxyl radicals and inhibited Fe^{2+}-induced MDA production in rat brain homogenate [35]. *Blighia sapida*, *Vitellaria paradoxa* and *Vitex doniana* did not only contain alkaloids, tannins, saponins, flavonoids and phenols but also showed dose-dependent inhibition of lipid peroxidation [36]. There are few reported anti-lipid peroxidation properties in the genus *Baccaurea.* The fruit *Baccaurea sapida* showed an inhibition of peroxide formation in a dose-dependent manner [37].

The antioxidant properties of the three different *B. angulata* WF juice doses indicate well-defined antioxidant effects of the juice. A study by Ling [38] showed that *Anacardium occidentale* Linn. at doses of 50 (0.05 ml/kg), 250 (0.25 ml/kg), 500 (0.50 ml/kg) and 1000 (1.00 ml/kg) mg/kg body weight was effective at increasing SOD activity in Type 2 rat model after 3 weeks of administration. A study revealed the protective effects of *Cornus mas* fruit extract on carbon tetrachloride (CCl_4)-induced cardiotoxicity in Albino rats. In the study, treatment of rats with the fruit extract at doses of 300 (0.30 ml/kg) and 700 (0.70 ml/kg) mg/kg significantly increased the activity of GPx [39]. In the present study, the effect was more pronounced in the rabbits of 1.50 ml/kg/day WF-treated group (C3) than the other two dose groups of animals (C2 and C1). Also, *B. angulata* WF at doses 0.50, 1.00 and 1.50 ml/kg/day demonstrated modulating effects on CAT activity as it did on GPx. Adebayo et al. [40] reported that from 0.50 (0.50 ml/kg), 1.00 (1.00 ml/kg) and 1.50 (1.50 ml/kg) g/kg body weight of extract groups of rats, the group treated with 1.50 g/kg body weight had significantly ($p < 0.05$) high CAT activity when compared with the positive control (hepatotoxin) group.

The result of the present study is also similar to the work of Vega-López et al. [41] who revealed that despite the somewhat high content of lycopene, α-carotene, β-carotene, α-tocopherol and γ-tocopherol in the soy protein-containing diets, the diets did not significantly increase TAC level.

The studies in rabbits show that diets high in saturated fat and cholesterol result in a condition characterized by the presence of high levels of cholesterol in the blood (hypercholesterolaemia). Furthermore, no bile salts and/or cholic acid drugs need to be added to rabbits' diet together with cholesterol to induce hypercholesterolaemia [42, 43]. Accordingly, in our unpublished study, rabbits demonstrated a marked response to a high-cholesterol diet by developing significant plasma cholesterol concentrations (CH 2.40 mol/l, C1 1.89 mol/l, C2 1.68 mol/l, C3 1.55 mol/l and N 0.02 mol/l).

The key contribution of hypercholesterolaemia to the increase in serum and aortic MDA and decrease in the antioxidant enzymes in aorta has been confirmed by many clinical trials and animal models. Prasad [44] reported an increase in aortic MDA and chemiluminescence in high cholesterol-fed rabbits compared with control group.

Hypercholesterolaemia in rats significantly decreased aorta and heart antioxidant enzymes (SOD, CAT, GPx and GST) activities [45]. Similarly, high-cholesterol diet has been reported to induce oxidative stress and to have negative effects on antioxidant enzymes in the liver [30, 46]. In this current study, high-cholesterol diet induced oxidative stress in rabbits by raising MDA levels in both aorta and liver. However, the administration to high cholesterol-fed rabbits of *B. angulata* WF juices at doses of 0.50, 1.00 and 1.50 ml/kg/day significantly ($p < 0.05$) prevented the elevation of MDA levels in the aorta and decreased the raised MDA levels in the liver compared to that of group CH. Our results indicate that *B. angulata* WF juices had a free radical scavenging activity which probably provides aorta and liver a protection from hypercholesterolaemia-enhanced MDA production. The protective effect of *B. angulata* WF juice is comparable with that of *Phyllanthus emblica* [47], *Phyllanthus amarus* [48] and *Bridelia ferruginea* [49], which belong to the same family. The preventive role of *B. angulata* is suggested to be due to its free radical scavenging activities and the abundant content of antioxidant phytochemical constituents (flavonoids, phenolic acids and phenolic diterpenes). The decrease in the activities of the antioxidant enzymes could be due to the fact that exposure to high cholesterol has been associated with down-regulation of the antioxidant enzymes biosynthesis [50]. An administration to high cholesterol-fed rabbits of *B. angulata* WF juices at doses of 0.50, 1.00 and 1.50 ml/kg/day prevented the reductions in the levels of SOD, GPx, CAT and TAC in the aorta and liver of rabbits. The effect was found to be dose-dependent for GPx and CAT in the aorta and SOD, GPx and TAC in the liver. It can be hypothesized that *B. angulata* WF juice responded to hypercholesterolaemia-enhanced free radical production with a pronounced up-regulation of the antioxidant enzymes in aorta and liver. A similar observation has been reported for *Tamarindus indica* L. pulp fruit extract [51], *Tulbaghia violacea* rhizome extract [45], chicory (*Cichorium intybus* L.) fruit extract [52] and extract of ajwain [53].

Conclusion

The present findings confirm the efficacy of *B. angulata* WF as a potential natural antioxidant and that the increasing dose of *B. angulata* WF juice exerted better antioxidant activity.

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Compliance with ethical standards

Conflict of interest Authors have no conflict of interests.

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