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Original Research

Growth rate, haematologic and atherogenic indicators of rats fed with cocoa beverages

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ABSTRACT

Background and Aim: The present study proposed that feeding experimental rats with processed cocoa bean-based beverages (PCB-BB) - and raw cocoa bean powder (RCBP) - containing diets could affect growth rate and probably, perturb blood homeostatic indices. **Materials and Methods:** The rat groups were designated on the basis of diets received for 28 days. The PCB-BB and RCBP were compounded separately with pelletized standard guinea feed (PSGF) (ratio 10:1 w/w) to obtain the experimental diets, whereas the control diet was composed of PSGF only. At the end of the feeding period, 12-hour post-fasted rat groups were measured for anthropometric indices before drawn blood samples were measured for haematologic parameters and serum lipid profile. **Results:** The weight gained by the rat groups ranged between 46.80±1.61 g and 53.83±1.87 g. Haematologic indicators of the rat groups were within relatively narrow ranges. Serum HDL-C concentrations of the rat groups were within the range of 0.7±0.2 mmol/L - 1.9±0.5 mmol/L. Serum VLDL-C concentrations of control and test groups showed no significant difference ($p > 0.05$). **Conclusions:** The comparable weight gained by the test rat groups was an indication that PCB-BB - and RCBP - containing diets met the minimum nutritional requirements for normal growth rate. Additionally, the consumption of the experimental diets did not elicit alterations in blood indicators that could have suggested the presence of systemic infections in the rats. Finally, the atherogenic indicators appeared to suggest that the consumption of PCB-BB - and RCBP - containing diets did not arrest atherogenesis following short-term feeding of the rats.

KEY WORDS: Atherogenesis; beverages; growth rate; serum lipid profile; *Theobroma cacao*.

INTRODUCTION

Constancy in internal environment of organisms, and by implication the vascular system, supposes that variations in blood physicochemical indices are kept within a narrow range but may exhibit substantial variations under pathologic states following exposure of the organism to noxious compounds or biological agents. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) are haematologic indicators for establishing inheritable blood disorders or exposure of the vascular system to haemolytic agents or noxious compounds that interferes with erythropoiesis and erythrocyte structural/functional integrity, and thereby elicit the anaemias. Serum lipid profile (SLP) describes the proportionate concentrations of circulating lipids in the vascular system. Alterations of SLP are characterized by hyperlipidemia, which is a multifaceted metabolic syndrome of primary genetic etiology or secondary causes associated with drug or dietary intakes and underlying pathophysiologic disorders [1-5]. Hyperlipidemia is linked with increased circulating levels of atherogenic lipoproteins in plasma, which is a risk factor and primary indicators of atherosclerosis and susceptibility to coronary heart disease [6,7].

Cocoa bean tree - *Theobroma cacao* (Linnaeus) originated from Latin America about 500 years ago and thereafter was propagated in Europe, from where the crop was introduced to other regions of the world [8,9]. It is a cash crop that is grown all over the humid tropical regions, which is made up of over 57 countries, in about 6.5 million hectares of cultivated land. The average worldwide yield of cocoa beans is approximately 400 kg/hectare/year. The world production of cocoa bean reached 2.7 metric million tons in 1998, with Africa holding a dominant 70% of production capacity, of which 40% and 15% came from Côte d'Ivoire and Ghana, respectively [10].

The quality parameters of cocoa beans from Nigeria, as well as cocoa bean-based beverages of different brands consumed in the Nigerian market, have previously been reported elsewhere [11-15]. Processed cocoa products exhibit alterations in physical attributes, loss of essential amino acid/antioxidant contents [16,17] and, possibly, microbial [15,18-22] and heavy metal [23,24] contaminations at many critical points during the production chains of processed cocoa bean-based beverages (PCB-BB), which in turn, determine the quality of the final products. Also, PCB-BB contains additives and preservatives to ensure increased shelf-life and improved organoleptic properties of the finished products. Some of these compounds and biological agents that are associated with the production scheme are assimilated into the body system after normal processes of digestion and may have substantial impact on human physiology, well-being and development.

There are several claims that the administration of cocoa products to experimental animal models promoted good health and well-being [25-31]; supporting the general belief that consumption of PCB-BB are nutritious. Fermentation and roasting of raw cocoa bean introduce adducts (the so-called phlobaphenes) and chemically modified biomolecules associated with the browning reactions [22,32-35]. There are

no available precise empirical data on the effect of PCB-BB, which are composed of Maillard reaction end-products and other chemically modified by-products [16,22,32-35] coupled with instances of heavy metal [23] and microbial contaminations [15,18,20-22], on physiologic blood indices and anthropometric parameters in animal models. Additionally, there are scanty information on the effects of consuming of raw cocoa bean meals on animal physiology and well-being due to the presence of microbial matter and actions of anti-nutrients in raw fermented cocoa bean.

Accordingly, the present study proposed that feeding experimental rats with diets containing PCB-BB commonly sold in Nigerian markets, which have undergone varieties of biological and physicochemical processing conditions and raw cocoa bean powder (RCBP), could affect growth rate, perturb blood homeostatic indices and probably, promote atherogenicity. The outcome of the present study will serve to reveal the suitability or otherwise of PCB-BB - and RCBP - containing diets for animal consumption, which hitherto may have been taken for granted.

MATERIALS AND METHODS

Collection and processing of raw cocoa-bean seeds

The cocoa-bean pods were randomly handpicked from small-holder cocoa farmers in Owerri, Imo State, Nigeria. The pods were harvested on the 24th September, 2014. The beans were evacuated from the pods and allowed to ferment for 5 days while shielded from sunlight. Fermentation of cocoa bean was done using the conventional heap fermentation method [36]. The wet beans were heaped on layers of plantain (*Musa paradisiaca*) leaves and covered with the same material to retain the heat generated during the fermentation process. On the third and fifth days, the beans were quickly and thoroughly re-mixed using a wooden spade and covered once again. Next, the fermented beans were sun-dried for ten days till constant weight was achieved. A 50 g sample of the beans were pulverized using Thomas-Willey milling machine (ASTM D-3182, INDIA), after which the ground samples were stored in air-tight plastic bottles with screw caps pending use to compound the rat diets.

Proximate composition and energy value of animal diets

The RCBP was mixed with sucrose (ratio 10:1 w/w) to sweeten it. The PCB-BBs were three (3) brands of cocoa beverages commonly consumed in Nigeria, which were purchased from a grocery shop. Also, PCB-BB and RCBP were compounded separately with PSGF (ratio 10:1 w/w) to obtain the test diets, whereas the control diet was composed of pelletized standard guinea feed (PSGF) only. The PSGF (product of a subsidiary of UAC Nigeria Plc., Jos, Nigeria) was purchased at the Relief Market, Owerri, Imo State, Nigeria. The proximate composition of RCBP and PSGF were measured by the methods of AOAC [37], whereas the energy value of the experimental diets were measured using the Atwater factor of 4, 9, 4 as described by Wardlaw and Kessel, [38]. The major composition and ingredients of the experimental diets according to manufacturers' descriptions or otherwise are summarized in Table 1.

Animal handling

The present study was approved by the Ethical Committee on the use of animals for research, Department of Biochemistry, Federal University of Technology, Owerri, Nigeria. The rats were obtained from the Animal House of the Department of Biochemistry, Federal University of Technology, Owerri, Nigeria. Female albino (Wistar) rats were maintained at room temperatures of 28±2 °C, 30–55% of relative humidity on a 12-h light/12-h dark cycle, with access to water and PSGF *ad libitum* for 2 weeks acclimatization period. Handling of the rats was in accordance with the standard principles of laboratory animal care of the United States National Institutes of Health (NIH, 1978). Ethics Approval Number: ODVC/REN/543/15.

Table 1. Descriptive major composition of feed/raw cocoa and ingredients of cocoa bean-based beverages

Sample	Descriptive composition/Ingredients
PSGF*	Cereals/grains, vegetable protein, vitamins, mineral, essential amino acids, salts, antioxidant, anti-toxins, prebiotic and enzymes.
RCBP	Ref: [16,23,29,39,40].
OT*	Barley-malt extract, milk, sugar, vegetable oil, minerals, salt, vitamins, vanillin, protein, cocoa powder, whey powder, natural color.
BV*	Malt extract, sugar, cocoa powder, glucose, skimmed milk, milk protein, emulsifier, minerals.
MO*	Sugar, milk, emulsifier, cocoa powder, vegetable oil, mineral, salt, protein, vitamins, soya.

*Manufacturers' information; PSGF = Pelletized standard guinea feed; RCBP = Raw cocoa bean powder; OT = Cocoa beverage 1; BV = Cocoa beverage 2; MO = Cocoa beverage 3.

Design of animal feed experiment

A total of 30 female Wistar rats (90 days old) of average weight of 106.0±2.0 g were allotted into five (5) groups of six (6) rats each. The rats were deprived of food and water for additional 16 h before commencement of feeding as described elsewhere [41]. The rat groups were designated on the basis of experimental diets received for 28 days.

- Group 1 (WR-PSGF): Wistar rats received PSGF + water *ad libitum*.
- Group 2 (WR-RCBP): Wistar rats received RCBP + water *ad libitum*.
- Group 3 (WR-OT): Wistar rats received OT + water *ad libitum*.
- Group 4 (WR-BV): Wistar rats received BV + water *ad libitum*.
- Group 5 (WR-MO): Wistar rats received MO + water *ad libitum*.

At the end of the feeding period, 12-hour post-fasted rat groups were measured for anthropometric indices before blood samples were drawn from the orbital sinus [42] and measured for haematologic parameters and serum lipid profile (SLP).

Feed consumption and growth rate

Anthropometric indices were measured as previously described [43]. The body weights of the experimental rats were measured before and after feeding period. The body weight gained at the end of the feeding period was calculated as the difference between the final and initial body weights. The quantity of feed consumed by the rats was calculated as the sum of the daily feed intakes throughout the experimental period, whereas feed conversion ratio was calculated as the ratio of the feed consumed to the body weight gained.

Haematology

Packed cell volume (PCV) was measured using the micro haematocrit methods. Measurement of haemoglobin (Hb) concentration was by the cyanomethaemoglobin methods [44] whereas; red blood cell (RBC), white blood cell (WBC) and platelet counts (PC) were measured using haemocytometer with improved neubauer slide [45]. Mean corpuscular haemoglobin (MCH) were calculated according to the methods of Jain, [46]. Differential leucocyte count (DLC), neutrophils count (NC) and eosinophil count (EC) were according to the methods of Feldman *et al*., [47].

Serum lipid profile

Experimental rat groups were measured for SLP according to the methods previously described [48]. Serum total cholesterol (TC), triacylglycerol (TAG) and high-density lipoprotein cholesterol (HDL-C) concentrations were measured using commercial kits (Randox Laboratory Ltd., UK). Low-density lipoprotein cholesterol (LDL-C) concentration was estimated according to the formula of Friedewald *et al*.,

$$\mathrm{LDL-C} = \mathrm{TC} - (\mathrm{HDL-C}) - \left(\frac{\mathrm{TAG}}{5}\right) \qquad Eq1$$

Accordingly, atherogenic indices of the experimental rat groups were calculated using standard formulae as previously described [43]. Atherogenic tendency was determined using the TAG/HDL-C ratio [51-53]. The log (TAG/HDL-C ratio) was a measure of atherogenic index of plasma (AIP) [53,54], whereas TC/HDL-C and LDL-C/HDL-C ratios were measures of atherogenicity [5].

Statistical analysis

The results were expressed as mean ± SEM, and statistically analyzed by one way ANOVA followed by Dunnett test, with level of significance set at $p < 0.05$.

RESULTS

The experimental diets were comparatively rich in carbohydrates; CV% = 20.33. Additionally, the energy values of the experimental diets were within the range of 196 - 403 kcal/100 g. The average fats and dietary fiber contents were comparable; except dietary fiber content of RCBP = 28.5 g/100 g, but were lower than the average protein contents of the experimental diets. Finally, the dietary characteristics of the various feeds, in terms of CV%, were in the order: dietary fiber =129.7% > fats = 48.18% > proteins > 36.62% > energy = 29.94% > carbohydrates = 20.33%

Table 2. Nutritional information of guinea feed, raw and processed cocoa bean-based beverages

Parameter	Guinea feed and cocoa derived beverages						
	PSGF#	RCBP	OT#	BV#	MO#	Mean	CV%
Energy (kcal/100 g)	255	196	413	359	403	319.2±95.557	29.94
Protein (g/100 g)	15	17	8	6.9	13	11.98±4.388	36.62
Carbohydrate (g/100 g)	NG	49.8	74	82	65.4	67.8±13.782	20.33
Fats (g/100 g)	7	11.8	9.4	1.9	8	7.62±3.671	48.18
Dietary fiber (g/100 g)	10	28.5	2.8	0.2	3	8.9±11.544	129.7

Values are means of six determinations ± S.D. #Manufacturers' descriptions; PSGF = Pelletized standard guinea feed; RCBP = Raw cocoa bean powder; OT = Cocoa beverage 1; BV = Cocoa beverage 2; MO = Cocoa beverage 3; CV = Coefficient of variation, NG = Not given.

The weight gained by the experimental rat groups ranged between 46.80±1.61 g and 53.83±1.87 g (Table 3). Notably, WR-RCBP represented the highest weight gained, whereas WR-BV exhibited the lowest weight gained among the various experimental rat groups. Specifically, the weight gained by WR-RCBP corresponded to an increase of 6.03% when compared with that of WR-PSGF. Additionally, WR-BV consumed the lowest quantity of feed. However, WR-BV exhibited the highest feed conversion ratio.

An overview of Table 4 showed that the variations in haematologic indicators of the experimental rat groups were within relatively narrow ranges. For instance, PCV of the rats was between 38.3±2.16% and 42.3±1.7%. Similarly, WBC was within the range of 74.3±6.87 cells/mL - 91.0±9.43 cells/mL.

Peak level of WBC was exhibited by WR-OT = 91.0±9.43 cells/mL. Amongst the various experimental rat groups, RBC was lowest in blood samples of WR-PSGF = 2.10±0.1 x10^9/mL, whereas that of WR-OT = 2.96±0.21 x 10^9/mL gave the highest RBC, which represented an increase in RBC by 40.95%. Levels of MCH amongst the experimental rat groups did not showed profound variations. Blood PC of WR-OT was 1.09 fold higher than that of WR-PSGF. Blood NC and DLC of the various experimental rat groups were 0.66±0.05 x 10^9/mL – 0.74±0.04 x 10^9/mL and 0.24±0.03 x 10^9/mL - 0.33±0.06 x 10^9/mL, respectively. Finally, blood samples of WR-RCBP, WR-OT and WR-BV showed traces of EC, whereas no EC was present in blood samples of WR-PSGF and WR-MO.

Figure 1 showed that serum TC concentrations of the test rat groups (WR-RCBP, WR-OT, WR-BV and WR-MO) were lower than that of the control group (WR-PSGF); $p < 0.05$. Specifically, serum TC concentration of WR-RCBP represented 16.32% reduction in serum TC concentration when compared with that of WR-PSGF. Serum TC concentrations of WR-OT, WR-BV and WR-MO were not significantly different ($p > 0.05$) and were equivalent to 1.32 folds lower than that of WR-PSGF.

Table 3. Anthropometry of experimental rat groups

Group	Weight gained (g)	Feed consumed (g)	Feed conversion ratio
WR-PSGF	50.77±2.94^{a,b}	632.5±2.91^{a}	12.46 ±0.03^{a,b}
WR-RCBP	53.83±1.87^{a}	630.2±1.94^{a,b,c}	11.71 ±0.61^{a,b,c,d}
WR-OT	47.98±2.07^{a,b,c,d}	540.9±2.17^{e}	11.27±0.53^{a,b,c,d,e}
WR-BV	46.80±1.61^{a,b,c,d,e}	590.7±1.17^{d}	12.62±0.52^{a}
WR-MO	50.62±3.44^{a,b,c}	625.6±3.15^{a,b}	12.36±0.56^{a,b,c}

Values are means of six determinations ± S.D. Values on the same column bearing the same superscript letter a, b, c, d, e are not significantly different ($p > 0.05$).

Table 4. Haematologic indicators of experimental rat groups fed with guinea feed, raw and processed cocoa bean-based beverages

	Haematologic indicators				
Parameter	WR-PSGF	WR-RCBP	WR-OT	WR-BV	WR-MO
PCV (%)	38.3±2.16	39.0±0.89	42.3±1.7	40.7±1.25	38.3±1.79
Hb mg/dL	13.0±0.72	13.3±0.31	14.4±0.54	13.9±0.33	13.5±0.82
WBC (Cells/mL)	74.3±6.87	81.3±7.30	91.0±9.43	85.3±7.3	84.0±5.53
RBC (10^9/mL)	2.10±0.10	2.40±0.41	2.96±0.21	2.67±0.26	2.48±0.24
MCH (ρg)	0.62±0.038	0.57±0.09	0.49±0.031	0.53±0.05	0.55±0.07
PC (10^9/mL)	1.98±0.45	2.15±0.29	2.16±0.54	2.01±0.2	2.00±0.17
NC (10^9/mL)	0.66±0.05	0.67±0.05	0.67±0.034	0.65±0.05	0.74±0.04
DLC (10^9/mL)	0.33±0.06	0.31±0.05	0.32±0.03	0.32±0.04	0.24±0.03
EC (10^9/mL)	0±0	0.002±0.001	0.001±0.001	0.002±0.001	0±0

Values are mean ± SD, PCV = Packed cell volume; Hb = Hemoglobin, WBC = White blood cell count; RBC = Red blood cell count; MCH = Mean corpuscular hemoglobin; PC = Platelet count; NC = Neutrophils count; DLC = Differential lymphocyte count; EC = Eosinophil count.

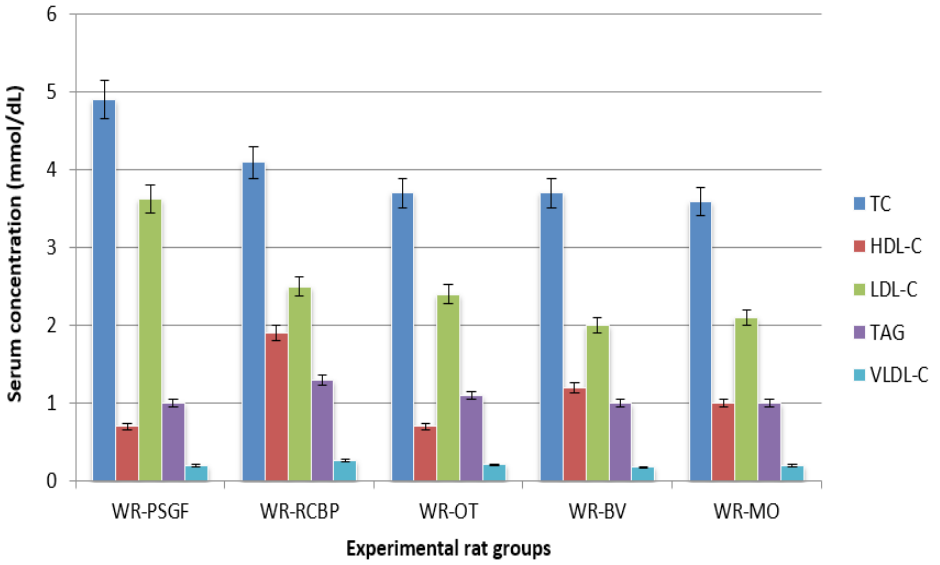

Figure 1. Serum lipid profiles of experimental rat groups fed with guinea feed, raw and processed cocoa bean-based beverages.

Serum HDL-C concentrations of the experimental rat groups were within the range of 0.7±0.2 mmol/L - 1.9±0.5 mmol/L. Serum HDL-C concentration of WR-RCBP gave peak value = 1.9±0.5 mmol/L, whereas the lowest serum HDL-C concentration was indicated in WR-PSGF and WR-OT. WR-PSGF exhibited the highest serum LDL-C concentration = 3.62±0.3 mmol/L. The comparatively low serum LDL-C concentrations of WR-RCBP, WR-OT, WR-BV and WR-MO were within the narrow range of 2.0±0.3 mmol/L - 2.5±0.2 mmol/L. Serum TAG concentrations of WR-PSGF, WR-RCBP, WR-OT, WR-BV and WR-MO were comparatively equivalent. Likewise, serum VLDL-C concentrations of WR-PSGF, WR-RCBP, WR-OT, WR-BV and WR-MO showed no significant difference ($p > 0.05$).

Table 5 showed that the atherogenic indicators (TC/HDL-C and LDL-C/HDL-C) of WR-RCBP, WR-OT, WR-BV and WR-MO were comparatively lower than that of WR-PSGF, whereas values of TAG/HDL-C indicator were within a narrow range of 0.68±0.56 - 1.57±0.25.

Additionally, WR-RCBPTAG/HDL-C = 0.68±0.56 represented the lowest atherogenic index of the experimental rat groups. A cursory look at Table 5 showed that WR-RCBP gave the lowest atherogenic indices (TAG/HDL-C = 0.68±0.56, TC/HDL-C = 2.16±0.34 and LDL-C/HDL-C = 1.32±0.59) when compared with other experimental rat groups.

DISCUSSION

The comparable weight gained by the various rat groups following the feeding experiment was an indication that the PSGF, RCBP, OT, BV and MO met the minimum nutritional requirements for normal growth rate, which depended on the quantity of feed consumed. In that order, the quantity of feed consumed by the rats also depended on the palatability of PSGF as well as that of PCB-BB - and RCBP - containing diets. Additionally, the feed conversion ratio of the experimental diets attested the comparable dietary quality and properties of PSGF, RCBP, OT, BV and MO as typified by the nutritional information of PCB-BB and RCBP (Tables 1 and 2).

Previous reports have shown that exposure of animals to toxic substances altered their blood composition and physiochemistry [55,56]. Precisely, the presence of certain anti-nutritional factors [57,58] and introduction of toxic substances in foods [59] could provoke haemolytic events, which are reflected by alterations in PCV, Hb and RBC values below lower limits of physiologic reference range in affected animals [60,61]. Hemolytic indicators (PCV% and Hb mg/dL) showed that the consumption of PSGF, PCB-BB - and RCBP - containing diets did not provoke hemolysis in the experimental rats groups. Rather, the outcome of the feeding experiment showed marginal increases of PCV, Hb and RBC values in WR-RCBP, WR-OT, WR-BV and WR-MO when compared with that of WR-PSGF. Furthermore, although the MCH values of WR-RCBP, WR-OT, WR-BV and WR-MO were comparatively lower than that of WR-PSGF, these values did not suggest the presentation of haemolytic anemia in rats fed with the corresponding experimental test diets. Likewise, the comparable WBC, PC, NC, DLC and EC values of the various experimental rat groups (Table 4) appeared to suggest narrow variations and probably, acceptable levels of microbial loads in the corresponding experimental diets. Previous reports have shown that microorganisms are normal constituents of cocoa beans and the outcome of mycological evaluation of raw and fermented cocoa beans could predict possible health hazards to consumers [15,21]. Overall, the present study showed that rats fed with PCB-BB - and RCBP - containing diets, in the present form, did not cause substantial alterations in haematologic indicators to warrant health hazards and pathogenic concerns.

Table 5. Atherogenic indicators of experimental rat groups

	Atherogenic indicators				
Groups	**TAG/HDL-C**	**log TAG/HDL-C**	**TC/HDL-C**	**log TC/HDL-C**	**LDL-C/HDL-C**
WR-PSGF	1.43±0.12	0.16±0.21	7.00±1.28	0.85±0.11	5.17±1.84
WR-RCBP	0.68±0.56	-0.17±0.25	2.16±0.34	0.33±0.24	1.32±0.59
WR-OT	1.57±0.25	0.20±0.07	5.29±1.60	0.72±0.12	3.43±1.42
WR-BV	0.83±0.50	-0.08±0.10	3.08±0.34	0.49±0.15	1.67±0.10
WR-MO	1.00±0.23	0.00±0.09	3.60±0.50	0.56±0.06	2.10±0.43

Values are means of six determinations ± S.D. Reference values: TAG/HDL-C ratio [51,53], TC/HDL-C < 1.66 and LDL-C/HDL-C ratios < 1.06 [5]; log TAG/HDL-C ratio < 0.11 [54].

The present study showed that WR-RCBP, WR-OT, WR-BV and WR-MO exhibited comparable SLPs patterns that were distinct from that of WR-PSGF (Figure 1). This finding was consistent with previous reviewed reports by Tokede *et al*., [30], in which they noted that flavanol-rich cocoa products promoted the maintenance of SLP within normal physiologic range. However, they further noted that cocoa products had no major effects on serum HDL-C and TAG concentrations following short-term intervention. In related studies, the consumption of raw garlic (*Allium sativum* L.) by diabetes mellitus/hyperlipidemic individuals during and after six-week treatment period [62] as well as administration of aqueous leaf extracts of *Ocimum sanctum* L. to streptozotocin-induced diabetic rats [63], caused the normalization of distorted SLPs within acceptable physiologic values. The reports above, in addition to other previous findings [48,64-66] corroborated the outcome of the present study in that the consumption of plant products promoted lipid homeostasis and, to a large extent, ameliorated disarrangement of blood lipid patterns in experimental animals. It is worthwhile to note that the similarity in SLPs patterns of WR-RCBP, WR-OT, WR-BV and WR-MO may not be unconnected with common organic contents of RCBP and PCB-BB. The high HDL-C score of WR-RCBP appeared to suggest that the consumption of RCBP caused relatively raised plasma levels of HDL-C, as previously reported [7,27], than the corresponding PCB-BB. The comparative higher capacity of RCBP to promote raised level of HDL-C than those of PCBB was probably as a result of alterations in the bioactive contents of cocoa beans following industrial processing as earlier proposed [16,22,32-35]. Generally, the WR-RCBP, WR-OT, WR-BV and WR-MO showed evidence of improved SLP patterns when compared with that of WR-PSGF.

By comparative analyses, WR-OT exhibited the highest atherogenic tendency as exemplified by the TAG/HDL-C ratio, which is a strong predicator of myocardial infarction and coronary disease [51-53], whereas serum lipid parameters indicated low AIP in WR-RCBP and WR-BV. Furthermore, according to Ibegbulem and Chikezie, [5] reference values (TC/HDL-C ratio < 1.66; LDL-C/HDL-C ratio < 1.06), the present results appeared to suggest that PCB-BB - and RCBP - containing diets exerted poor control on atherogenicity, and probably, the prevention of thrombotic events following short-term intervention measures as earlier described [30]. In the same manner, rats fed with experimental diet of comparatively high in fibre content ([RCBP] = 28.5 g/100 g; Table 2) did not exhibit low atherogenic indices following the short-term investigation. The apparent failure of RCBP to deter atherogenesis, on basis of short-term feeding experiment, may not be unconnected with the more impact that adjustments in metabolic events bring to bear to retard atherogenesis, rather than dietary fibre facilitated elimination of atherogenic substrates from the alimentary canal prior to their uptake into system circulation. For instance, the administration of primary inhibitors of cholesterol biosynthesis (fibric acid derivatives such as clofibrate) caused marked decrease in serum lipoproteins within 12 hours and 4 days in experimental rats [67]. Nevertheless, the atherogenic indictors of WR-RCBP were marked improvement over that of WR-OT, WR-BV, WR-MO and WR-PSGF.

CONCLUSION

The comparable weight gained by the test rat groups following the feeding experiment was an indication that PCB-BB - and RCBP - containing diets met the minimum nutritional requirements for normal growth rate. Additionally, the consumption of PCB-BB - and RCBP - containing diets did not elicit alterations in blood indicators that could have suggested the presence of systemic infections in the rats. The experimental rats groups fed with PCB-BB- and RCBP - containing diets exhibited comparable SLP patterns that were distinct from that of the WR-PSGF. The SLPs of WR-RCBP, WR-OT, WR-BV and WR-MO appeared to be an improvement over that of WR-PSGF. To a large extent, WR-PSGF exhibited comparative high level of atherogenicity amongst other experimental rat groups. Finally, the atherogenic indicators appeared to suggest that the consumption of PCB-BB - and RCBP - containing diets did not arrest atherogenesis following short-term feeding of the rats.

SOURCE OF SUPPORT

Nil

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Raasch RH. Hyperlipidemias. In: Applied Therapeutics. The Clinical Use of Drugs (Young LY, Koda-Kimble MA. (Eds). Edwards Brothers, Ann Arbor, MI. 1988; pp. 1743–1760.
2. Eliot LA, Jamali F. Pharmacokinetics and pharmacodynamics of Nifedipine in untreated and atorvastatin-treated hyperlipidemic rats. J Pharmacol Exp Ther. 1999; 291:188–193.
3. El-Demerdash FM, Yousef MI, Abou El-Naga NI. Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats. Food Chem Toxicol. 2005; 43:57–63.
4. Georgiadis AN, Papavasiliou EC, Lourida ES, Alamanos Y, Kostara C, Tselepis AD, Drosos AA. Atherogenic lipid profile is a feature characteristic of patients with early rheumatoid arthritis: effect of early treatment – a prospective, controlled study. Arthritis Res Ther. 2006, 8:R82. 7pages.
5. Ibegbulem CO, Chikezie PC. Serum lipid profile of rats (Rattus norvegicus) fed with palm oil and palm kernel oil-containing diets. Asian J Biochem. 2012; 7:46–53.
6. Fuhrman B, Rosenblat M, Hayek T, Coleman R, Aviram M. Ginger extract consumption reduces plasma cholesterol, inhibits LDL oxidation and attenuates development of atherosclerosis in atherosclerotic, apolipoprotein E-deficient mice. J Nutr. 2000; 130:1124–31.
7. Khan N, Monagas M, Andres-Lacueva C, Casas R, Urpí-Sardà M, Lamuela-Raventós RM, Estruch R. Regular consumption of cocoa powder with milk increases HDL cholesterol and reduces oxidized LDL levels in subjects at high-risk of cardiovascular disease. Nutr Metab Cardiovasc Dis. 2012; 22:1046–1053.
8. International Cocoa Organization (ICCO) - Annual Report 1997/1998 2000; 1–4.
9. Chin E, Miller KB, Payne MJ, Hurst WJ, Stuart DA. Comparison of antioxidant activity and flavonol content of cacao beans processed by modern and traditional Mesoamerican methods. Herit Sci. 2013; 1:9.
10. Omont H. Importance of Cocoa. IPGRI – Commodity Chains. 2001; pp.1–4.
11. Olaofe O, Oladeji EO, Ayodeji OE. Metal contents of some cocoa beans produced in Ondo State, Nigeria. J Sci Food Agric. 1987; 41:241–244.
12. Oluwafemi F, Ayanwande B. Ochratoxin A in Cocoa beans and cocoa based beverages in Nigeria: Traceability from farmgate to export outlet. Nig Food J. 2008; 26(1):http://dx.doi.org/10.4314/nifoj.v26i1.47423
13. Oke DO, Omotayo KF. Effect of forced-air artificial intermittent drying on cocoa beans in South-Western Nigeria. J Cereal Oil Seed. 2012; 3:1–5.
14. Ogah CO, Obebe OT. Caffeine Content of Cocoa and Coffee Beverages in Lagos, Nigeria. J Innov Res Eng Sci. 2012; 3:404–411.
15. Oyetunji TO. Mycological evaluation of a ground cocoa-based beverage. Afr J Microbiol. 2013; 1:33–36.
16. Adeyeye EI, Akinyeye RO, Ogunlade I, Olaofe O, Boluwade JO. Effect of farm and industrial processing on the amino acid profile of cocoa beans. Food Chem. 2010; 118:357–363.
17. Schinella G, Mosca S, Cienfuegos-Jovellanos E. Antioxidant properties of polyphenol-rich cocoa products industrially processed. Food Res Int. 2010; 43:1614–1623.
18. Tortajada M, Martínez-Culebras PV, Navarro V, Monzó H, Ramón D. Evaluation of DNA extraction methods for PCR detection of fungal and bacterial contamination in cocoa extracts. Eur Food Res Technol. 2009; 230:79–87.
19. Turcotte A-M, Scott PM. Ochratoxin A in cocoa and chocolate sampled in Canada. Food Addit Contam. 2011; 28:762–766.
20. Jayeola CO, Oluwadun A, Yahaya LE, Dongo LN, Ajao AA, Mokwunye FC. Comparative analysis of detecting ochratoxin A in cocoa powder samples using high performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA). Afr J Food Sci. 2011; 5:513–521.
21. Copetti MV, Iamanaka BT, Pereira JL, Lemes DP, Nakano F, Taniwaki MH. Determination of aflatoxins in by-products of industrial processing of cocoa beans. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2012; 29:972–978.
22. Stadler RH, Hughes G, Guillaume-Gentil O. Safety of food and beverages: Coffee, tea and herbals, cocoa and derived products. Food Mater Technol Risk. 2014; 3:371–383.
23. Rankin CW, Nriagu JO, Aggarwal JK, Arowolo TA, Adebayo K, Flegal AR. Lead contamination in cocoa and cocoa products: Isotopic evidence of global contamination. Environ Health Perspect. 2005; 133:1344–1348.
24. Russell FA. Lead contamination in cocoa and cocoa products: isotopic evidence of global contamination. Environ Health Perspect. 2005; 113:1344–1348.
25. Eteng MU, Ettarh RR. Comparative effects of theobromine and cocoa extract on lipid profile in rats. Nutr Res. 2000; 20:1513–1517.
26. Karim M, McCormick K, Kappagoda CT. Effects of cocoa extracts on endothelium-dependent relaxation. J Nutr. 2000; 130:2105S–2108S.
27. Ruzaidi A, Amin I, Nawalyah AG, Hamid M, Faizul HA. The effect of Malaysian cocoa extract on glucose levels and lipid profiles in diabetic rats. J Ethnopharmacol. 2005; 98:55–60.
28. Jalil AM, Ismail A, Pei CP, Hamid M, Kamaruddin SH. Effects of cocoa extract on glucometabolism, oxidative stress, and antioxidant enzymes in obese-diabetic (Ob-db) rats. J Agric Food Chem. 2008; 56:7877–7884.
29. Ruzaidi AM, Abbe MM, Amin I, Nawalyah AG, Muhajir H. Protective effect of polyphenol-rich extract prepared from Malaysian cocoa (Theobroma cacao) on glucose levels and lipid profiles in streptozotocin-induced diabetic rats. J Sci Food Agric. 2008; 88:1442–1447.
30. Tokede OA, Gaziano JM, Djoussé L. Effects of cocoa products/dark chocolate on serum lipids: a meta-analysis. Eur J Clin Nutr. 2011; 65:879–886.
31. Ibero-Baraibar I, Abete I, Navas-Carretero S, Massis-Zaid A, Martinez JA, Zulet MA. Oxidised LDL levels decreases after the consumption of ready-to-eat meals supplemented with cocoa extract within a hypocaloric diet. Nutr Metab Cardiovasc Dis 2014; 24:416–422.
32. Dauda WRW, Taliba MZM, Kyib TM. Drying with Chemical Reaction in Cocoa Beans. Drying Technol Int J. 2007; 25:867–875.
33. Farah DMH, Zaibunnisa AH, Misnawi J, Zainal S. Effect of roasting process on the concentration of acrylamide and pyrizines in roasted cocoa beans from different origins. APCBEE Procedia. 2012; 4:204–208.
34. Jumnongpon R, Chaiseri S, Hongsprabhas P, Healy JP, Meade SJ, Gerrardb JA. Cocoa protein crosslinking using Maillard chemistry. Food Chem. 2012; 134:375–380.
35. Krysiak W, Adamski R, Zyzelewicz D. Factors affecting the color of roasted cocoa bean. J Food Qual. 2013; 36:21–31.
36. Fapohunda SO, Afolayan A. Fermentation of cocoa beans and antimicrobial potentials of the pod husk phytochemicals. J Phys Pharm Adv. 2012; 2:158–164.
37. AOAC (Association of Official Analytical Chemists), 2006. Official Method of Analysis of the AOAC (W. Horwitz, Ed) 18th edn. Washington D.D.
38. Wardlaw GM, Kessel MW. Perspective in nutrition, 5th Edition. McGraw-Hill, Boston. 2002.
39. Subhashini R, Rao USM, Sumathi P, Gunalan G. A comparative phytochemical analysis of cocoa and green tea. Indian J Sci Technol. 2010; 3:188–192.
40. Minife BW. Chocolate, cocoa and confectionary (3rd Ed.). London: Chapman and Hall. 1989.
41. Ibegbulem CO, Chikezie PC. Hypoglycemic properties of ethanolic extracts of Gongronema latifolium, Aloe perryi, Viscum album and Allium sativum administered to alloxan-induced diabetic albino rats (Rattus norvegicus). Pharmacogn Commun. 2013; 3:12–16.
42. Hoff N. Methods of blood collection in the mouse. Lab Ani. 2000; 29:47-53.
43. Ibegbulem CO, Nwaogu LA, Udoala US, Belonwu DC, Udechukwu CN. Low dietary fiber causes low-risk atherogenicity in male Wistar rats fed a cereal-containing infant formula. J Invest Biochem. 2014; 1:1–5.
44. Baure JD. Laboratory investigation of hemoglobin. In: Gradwohl's Clinical Laboratory Methods and Diagnosis, (Eds) Sonnenwirth AC, Jarett L. St. Louis, MO: Mosby. 1980.
45. Douglas W, Harold T. The complete blood count and bone marrow examination: general comments and selected techniques. 4th Edn. Saunders and Elsevier Publisher, Philadelphia, Pennsylvania, USA. 2004; pp. 32–33.
46. Jain NC. Schalm's veterinary hematology. Lea and Febiger. 1986; pp. 276–282.

47. Feldman BF, Zinkl JG, Jain NC. Schalm's Veterinary Hematology. 5th ed., Lea and Febiger, Philadelphia, USA. 2000.
48. Ojiako AO, Chikezie PC, Zedech UC. Serum lipid profile of hyperlipidemic rabbits (Lepus townsendii) administered with leaf extracts of Hibiscus rose-sinesis, Emilia coccinea, Acanthus montanus and Asystasia gangetica. J Med Plant Res. 2013; 7:3226–3231.
49. Friedewald W, Levy R, Fredrickson D. Estimation of concentration of low-density lipoprotein in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18:499–502.
50. Shaker E, Mahmoud H, Mnaa S. Silymarin. The antioxidant component and Silybum marianum extracts prevent liver damage. Food Chem Toxicol. 2010; 48:803–806.
51. Gaziano JM, Hennekens CH, O'Donnell CJ, Breslow JL, Buring JE. Fasting triglycerides, high-density lipoprotein, and risk of myocardial infarction. Circ.1997; 96: 2520–2525.
52. da Luz PL, Favarato D, Faria-Neto JR Jr, Lemos P, Chagas AC. High ratio of triglycerides to HDL-cholesterol predicts extensive coronary disease. Clin (Sao Paulo). 2008; 63:427–432.
53. Bittner V, Johnson D, Zineh I, Rogers WJ, Vido D, Marroquin OC, Bairey-Merz CN, Sopko G. The triglyceride/high-density lipoprotein cholesterol ratio predicts all-cause mortality in women with suspected myocardial ischemia: A report from the Women's Ischemia Syndrome Evaluation (WISE). Am Heart J. 2009; 157:548–555.
54. Dobiasova M, Frohlich J. The plasma parameter log (TG/HDL-C as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apoB-lipoprotein-depleted plasma (FERHDL). Clin Biochem. 2001; 34:583–588.
55. Chua HP, Murugaiyah M, Rohani MY, Aminah A. Toxicological evaluation of dried kacangma (Leonurus sibiricus) in rats: I. Blood chemistry, body and organ weight changes. J Trop Agric Food Sci. 2006; 34:57–65.
56. Michałowicz J, Duda W. Phenols–Sources and Toxicity. Pol J Environ Stud. 2006; 16:347–362.
57. Liener IE. Toxic factors in edible legumes and their elimination. Am J Clin Nutr. 1962; 11:281–298.
58. Enneking D, Wink M. Towards the elimination of antinutritional factors in grain legumes. In: Knight R. (ed.) Linking Research and Marketing Opportunities for Pulses in the 21st Century. Proceedings of the Third International Food Legume Research Conference, Adelaide 1997. Current Plant Science and Biotechnology in Agriculture. Vol. 34. Kluwer Academic Publishers, Dordrecht/Boston/London. 2000; pp. 375–384.
59. Mundaw R, Manns E. Comparative toxicity of prop(en)yl disulfides derived from Alliaceae: possible involvement of 1-propenyl disulfides in onion-induced hemolytic anemia. J Agric Food Chem. 1994; 42:959–962.
60. Munday R, Smith BL, Fowke EA. Haemolytic activity and nephrotoxicity of 2-hydroxy-1, 4-naphthoquinone in rats. J Appl Toxicol. 1991; 11:85–90.
61. Ukpabi UH, Ukpabi UJ. Potential of seeds of Napoleona imperialis (p. beauv) as a source of haemolytic saponin and feed ingredients. Livest Res Rural Dev. 2003; 15:6pages.
62. Mahmoodi M, Hosseini Zijoud SM, Hassanshahi GH, Toghroli MA, Khaksari M, Hajizadeh MR, Mirzajani E. The effects of consumption of raw garlic on serum lipid level, blood sugar and a number of effective hormones on lipid and sugar metabolism in hyperglycemic and/or hyperlipidemic individuals. Adv Biol Chem. 2011; 1:29–33.
63. Suanarunsawat T, Ayutthaya WDN, Songsak T, Thirawarapan S, Poungshompoo S. Lipid-lowering and anti-oxidative activities of aqueous extracts of *Ocimum sanctum* L. leaves in rats fed with a high-cholesterol diet. Oxid Med Cell Longev. 2011; Article ID 962025, 9pages.
64. Sadeek EA, Hala, El-Rahman AA, Ali WS. The Effect of green roasted and decaffeinated coffee on serum glucose, insulin and serum lipid profile in diabetic rat models. J Am Sci. 2010; 6:968–969.
65. Abuelgassim OA. Effect of Acacia nilotica fruit extract on serum glucose and lipid concentrations in alloxan-induced diabetic rats. Pak J Biol Sci. 2013; 16:1398–1402.
66. Ojiako AO, Chikezie PC, Ogbuji CA. Blood glucose level and lipid profile of alloxan-induced hyperglycemic rats treated with single and combinatorial herbal formulations. J Tradit Compl Med. 2015; DOI: 10.1016/j.jtcme.2014.12.005.
67. Segal P, Roimim PS, Eder HA. Effect of Clofibrate on lipoprotein metabolism in hyperlipidemic rats. J Clin Invest. 1972; 51:1632–1638.

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