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Effect of Garcinia Kola Consumption on Lipid Profile and Body Weight of Rats Fed with High Fat Diet

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MMB, NNT, MGA and ERA conceptualized the study and performed methodology. Authors MMB, NNT and DJF investigated the work. Authors MMB, MGA and DJF did the formal analysis. Authors NNT and ERA did the project administration. Author NNT did software analyses, data curation and resources, validation. Author ERA did data visualization. Authors ERA, TNL and MCMF did data validation and supervised the study. Authors MMB, NNT and MGA wrote original draft. Authors NNT, DLF, TNL and MCMF wrote, reviewed and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Bitter kola (*Garcinia kola*) is commonly consumed as a snack in many localities in Cameroon and is considered to have health properties including weight-reducing agent by many people. This study aimed to evaluate the potential of bitter kola in weight management and prevention of obesity.

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Study Design: An experimental full 3×3 factorial design was used for this study, with the main responses being the anthropometric and biochemical parameters of the albinos Wistar rats.
Place and Duration of Study: The study was carried out at the Food Science Laboratory of the Department of Nutrition, Food and Bioresource Technology of the College of Technology, The University of Bamenda, Cameroon, between February and July 2023.
Methodology: Three bitter kola cultivars (*Bafia, Penja, Widikum*) were collected from their production areas, and their phytochemical content and antioxidant activity were analysed. The effect of their dry powder consumption on body weight and lipid profile was investigated on male Wistar rats consuming a high-fat diet. The rats were allocated to 10 groups of 6 rats including one control with the feed containing 0 g/kg of bitter kola powder, and 3 test groups per cultivar having 5 g, 10 g, and 15 g of bitter kola powder per kg of feed respectively.
Results: Results revealed that antioxidant activity varies with cultivars and W*idikum* cultivar has the highest antioxidant activity and the highest phytochemicals content. Consumption of *Penja* and *Widikum* bitter kola powder induced the highest fat excretion in rat faeces. The effect of bitter kola consumption on body weight varies with cultivars and incorporation rate, and the highest anti-obesity effect was observed with the consumption of the *Widikum* cultivar at 10 g/kg of rat feed. The increase in bitter kola powder proportion in rat feed was negatively correlated with the atherogenic index of plasma and atherogenic coefficient.
Conclusion: From the overall results, the consumption of feed with 15 g/kg of *Widikum* bitter kola cultivar had the best anti-obesity activity and hypolipidemic effect. Thus, bitter kola powder can be used to prevent obesity and cardiovascular diseases in high-fat diets.

Keywords: Garcinia kola; high-fat diet; wistar rats; anti-obesity activity; serum lipid profile; atherogenic index.

1. INTRODUCTION

Obesity is a complex disorder caused by the interaction of numerous genetic, dietary, lifestyle, and environmental factors [1]. Generally, obesity is defined as abnormal or excessive fat accumulation that may impair health. Obesity is associated with increasing health and societal costs and is a major public health issue. Once considered a high-income country problem, overweight and obesity are now on the rise in low- and middle-income countries, mainly in populations living in urban areas. Worldwide, the prevalence of obesity is rising with the highest rate in low- and middle-income countries, and this prevalence has nearly tripled since 1975 [2]. More than 1.9 billion adults (39% of adults) (18 years and older) are overweight, with over 650 million (13% of adults) being obese. In addition, over 340 million children and adolescents aged 5–19 are overweight or obese, and 38 million children under the age of 5 are overweight or obese, increasing the risk of development of cardiovascular diseases in their adulthood [3]. In Sub-Saharan Africa, overweight has been on the increase in all regions since 1990, although the extent of the increase varies between regions [2].

Obesity and overweight mainly result from an energy imbalance between calories intake and calories expended [4]. Globally, there has been an increased intake of energy-dense foods that are high in fat and sugars; and a decrease in physical activity due to the increasingly sedentary nature of many forms of work, changing modes of transportation, and increasing urbanisation. The comorbidities associated with obesity include type-2 diabetes, cardiac diseases, hypertension, sleep apnea, cerebrovascular incidents, osteoarthritis, and certain types of cancers [5]. Thus, it is important to manage, control, and reduce the rate of obesity and its comorbidities.

Obesity and overweight can be reduced at the individual level by reducing energy intake from lipids and carbohydrates, increasing consumption of food rich in fibre like fruits and vegetables, as well as legumes, whole grains and nuts, and engaging in regular physical activity [6,7]. Due to the constraining nature of these methods, many conventional drugs have also been used for the prevention and management of obesity in recent times [8]. The utilisation of these drugs is limited by their availability and harmful side effects. As a result, natural substances most especially plant sources have developed as anti-obesity agents with easy availability and minimal side effects. The wide variety of anti-obesity natural agents presents in plants or their extracts acts through various

mechanisms to either prevent weight gain or induce weight loss [9].

Natural anti-obesity products are categorised based on the mechanisms of action through which they exert their activity, these mechanisms include nutrient digestion and absorption inhibitors, enzymes (lipase and amylase) inhibitors, appetite and hunger suppressants and/or satiety inducers, stimulants of energy expenditure, modulators of the adipocytes life cycle, regulators of lipid metabolism, or substances with multifunctional anti-obesity activity [8]. Natural anti-obesity products are mostly complex in terms of chemical composition and the main active components are dietary fibre and phytochemicals. One of the important sources of these molecules is bitter kola (*Garcinia kola*) nuts [10], consumed in many localities in Cameroon as a natural snack and considered by many consumers as a hunger suppressant. Indeed, bitter kola nuts is used in many Africans indigenous medicine and studies have shown that they are good sources of several important classes of bio-actives molecules like biflavonoids, benzophenones, benzofurans, benzopyran, vitamin E derivatives, xanthones, and phytosterols and many other important phytochemicals [11]. Despite its high phytochemical content, there is no scientific evidence on the anti-obesity activity of the varieties of the bitter kola nut locally consumed in Cameroon. Thus, this work has as a general objective to evaluate the anti-obesity properties of bitter kola nuts in a high-fat diet.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Three cultivars of Bitter kola nuts were bought from the various local markets, Bamenda town food market (5°57’41” N, 10°9’6” E) for *Widikum* cultivars, Penja market (4°37’59.99” N, 9°40’59.99” E) for *Penja* cultivar and Bafia market (4°45’00” N, 11°13’00” E) for *Bafia* cultivar. The nuts were sorted to remove all foreign material and blemish nuts. The outer brown coat of the nuts was peeled off manually and the nuts were washed with tap water and allowed to drain in a colander. The drained nuts were spread on an oven tray and dried in an oven (DHG-9101-1SA, Searchtech instruments SANFA, China) at 45 °C for 24 h. The dried nuts were allowed to cool then ground to powder using a blender (Preethi Zodiac, India), sieved to obtain flour using a sieve of pores size of 1000 µm, packed in a high-density polyethylene back, and kept in an airtight glass container at room temperature (22 ±3 °C) for further uses.

2.2 Chemical and Antioxidant Analysis of the Bitter Kola Seeds Powder

Before flour production, the moisture content of the bitter kola samples was measured using the AOAC [12] method. For the dried powder, crude fibres content was determined using the method of Wolff [13], total protein content was obtained from nitrogen content after digestion and titration [14], and ash, crude fat, and total carbohydrates contents were determined using AOAC [12] methods. The phytochemicals, mainly alkaloids, total phenols, total tannins, total flavonoids, and saponin contents were determined using Harborne [15], Marigo [16], Makkar et al. [17], Siddhuraju and Becker [18] and Kozioł [19] methods respectively. The antioxidant activity was evaluated based on DPPH radical scavenging activity [20] with sample concentration varying from 100 µg/mL to 1000 µg/mL and the gallic acid used as standard, with concentration varying from 10 µg/mL to 100 µg/mL. The antioxidant activity was expressed using the IC_{50} value corresponding to the amount of antioxidants necessary to halve the initial DPPH concentration.

2.3 Effect of Bitter Kola Consumption on Body Weight and Serum Lipid Profile

2.3.1 Diet formulation and animal study

For the anti-obesity activity evaluation, male Wistar albi-nos’ rats of 8 weeks old (150–200 g) from the Department of Biochemistry of the Faculty of Science, University of Ya-oundé 1, Cameroon were used. The animals were randomly divided into 10 groups of 6 rats each. The rats were kept in the cages at 22 °C ±3 °C (room temperature) with wood shaving as litter, with a cycle of 12 h light and dark cycles and the water was given ad libitum. The rats were acclimatised for two weeks and fed during that period with a standard diet (car-bohydrates 65–70%, fat 30–35%, proteins 10–15%; 3810 kcal/kg) [21]. After acclimatisation, the rats were fed for 3 weeks on a High Fat Diet (HFD) of 7160 kcal/kg [22] con-taining bitter kola powder at different proportions (Table 1).

During the animal study, the control group was fed with HFD without bitter kola powder. For the test groups, the first (*Bafia* A), the second (*Bafia* B), and the third (*Bafia* C) groups were fed with HFD containing 5 g, 10 g, and 15 g of *Bafia* bitter kola powder per kilogram of feed respectively; group 4 (*Penja* A), group 5 (*Penja* B) and group 6 (*Penja* C) were fed with HFD containing 5 g, 10 g and 15 g of *Penja* bitter kola per kilogram of feed of powder respectively; group 7 (*Widikum* A), group 8 (*Widikum* B) and group 9 (*Widikum* C) were fed with HFD containing 5 g, 10 g and 15 g of *Widikum* bitter kola per kilogram of feed respectively. The weight of the animal was recorded every 3 days, the feed intake was recorded daily. After three weeks of feeding, the rats were fasted overnight, chloroform impregnated on clean and sterile cotton ball was used to anaesthetise the animals by inhalation in a sealed acrylic plastic chamber following loss of righting reflex and reduction in cardiac rate for about 2 min. The blood of the anaesthetised rats was collected through cardiac puncture. The weight of the organs (liver, kidneys, heart, and, spleen) was recorded for each rat, and the organ index was calculated (1). The animal study was carried out following ARRIVE guidelines [23].

$$\text{Organ index} = \frac{Net\ organ\ weight\ (g)}{Body\ weight\ (g)} \times 100 \qquad (1)$$

2.3.2 Lipid profile analysis and atherogenic index determination

To determine the effect of bitter kola powder consumption on blood lipid profile, the blood was collected in the dry tubes and centrifuged at 3500 rpm for 15 min to collect the serum. The triglycerides, the total cholesterol, and the high-density lipoprotein (HDL) were determined in the serum using Richmond [24] and Glick et al. [25] methods and the low density lipoprotein (LDL) was determined by calculation according to the Friedewald et al. [26].

2.3.3 Determination of faecal lipid content and atherogenic indices

The faeces were collected daily, dried, and crushed with the porcelain mortar into fine powder to evaluate the total lipid excreted by Bourely [27] method. The atherogenic index of plasma (AIP) (3) and atherogenic coefficients (AC) (4) were calculated [28].

AIP = Log (serum triglyceride/serum HDL). (2)

Atherogenic coefficient (AC) =

(Total cholesterol-HDLc)/HDLc, (3)

2.4 Statistical Analysis

An experimental full 3×3 factorial design was used for this study. The analysis was carried out in triplicate and the results were presented as means with their standard deviation (means ±SD). Analysis of variance (ANOVA) was performed to test the effect of cultivar and proportion on each response. Duncan's multiple range test was used to compare the means. The Pearson correlation test was performed to evaluate the relation between the different variables. The principal component analysis was performed to find the main components affecting the antioxidant activity of the bitter kola powder. The statistical analyses were performed using the Statgraphics Centurion software, version 17 with $P \leq .05$.

Table 1. Formulation of a high-fat diet for rats

Nutrient/bitter kola	Ingredient (g)	Control	Group A	Group B	Group C
Protein	Fish powder	140	140	140	140
Carbohydrates	Starch	250	250	250	250
Lipids	Coconut oil	250	250	250	250
	Egg yolk	250	250	250	250
	Soya bean oil	50	50	50	50
Minerals	Minerals	10	10	10	10
Vitamins	Vitamins	50	50	50	50
Bitter kola	Bitter kola powder	0	5	10	15

3. RESULTS AND DISCUSSION

3.1 Composition of the Different Bitter Kola Cultivars

3.1.1 Proximate composition of the different cultivars of bitter kola

The proximate composition of 3 cultivars of bitter kola obtained from Bafia, Penja, and Widikum were analysed and the results are presented in Table 2. The moisture content of the powder varies from 10.80% for the *Widikum* cultivar to 13.34% for the *Penja* cultivar. This variation could be due to the difference in chemical composition, making some samples more hygroscopic, or to the environmental conditions during production, harvesting, and storage. Similar results were reported by Onyekwelu et al. [29] on the bitter kola collected from Nigeria. Bitter kola nuts powder analysed contains mainly carbohydrates from 63.64 g/100 g DM for the *Bafia* cultivar to 68.69 g/100 g DM for *Widikum* cultivar. The ash content varies from 1.62 g/100 g DM (*Bafia*) to 1.95 g/100 g DM (*Widikum*) and is lower than the values reported by Ebana et al. [30] on seeds of *Dacryodes edulis* (9.62%) and *Garcinia kola* (10.81%).

Bitter kola nuts have analysed globally low protein content, varying significantly with the cultivar from 4.47 g/100 g DM (*Widikum*) to 5.74 g/100 g DM (*Bafia*). These results are similar to those reported by Odebunmi et al. [28] in *G. kola* nuts from Nigeria. However, the protein content obtained in this study is lower than the value reported in bitter kola seeds from Nigeria [30].

The lipid content ranging from 5.76 g/100 g DM (*Penja*) to 7.82 g/100 g DM (*Bafia*) is similar to the value reported in Nigeria samples of bitter kola [30]. Bitter kola nuts analysed are good sources of fibre (Table 1). The fibre content varies with cultivar and ranges from 20.33 g/100 g DM (*Widikum*) to 23.18 g/100 g DM (*Bafia*). These values are higher than those reported by Onyekwelu et al. [29] (3.65%) and Odebunmi et al. [31] (5.23%) in bitter kola nuts.

The difference in proximate composition between the different cultivars as well as between the results obtained in this study and those of other authors could be a result of differences in agroecological areas with different types of soils and climates, the maturity of the seeds, the varieties or the initial moisture content of the seeds analysed.

3.1.2 Phytochemicals content and antioxidant activity of the bitter kola cultivars

The phytochemical contents of bitter kola powder obtained from Bafia, Penja, and Widikum are presented in Table 2. Bitter kola contains many phytochemicals and their contents vary significantly ($p \leq 0.05$) with cultivars. The alkaloid content ranges from 933.33 mg/100 g DM (*Widikum*) to 1967.67 mg/100 g DM (*Bafia*) and is higher than the results reported by Onyekwelu et al. [29] in the same seeds (139 mg/100 g). The saponin content varies significantly with varieties and ranges from 1050.01 mg/100 g DM (*Penja*). to 2557.67 mg/100 g DM (*Widikum*). The polyphenol content ranges from 299.33 mg/100 g DM (*Widikum*) to 521.67 mg/100 g DM (*Bafia*) and the tannin content ranges from 103 mg/100 g DM (*Penja*) to 167.77 mg/100 g DM (*Widikum*). Polyphenols are functional compounds that have anti-carcinogenic, antioxidant, antibacterial, and antiviral activities [32].

Table 2. Chemical composition and antioxidant activity of bitter kola powder

Samples	Bafia	Penja	Widikum
Moisture (%)	12.40 ± 0.01^{b}	13.34 ± 0.03^{c}	10.80 ± 0.02^{a}
Carbohydrates (g/100 g DM)	63.64 ± 1.12^{a}	67.29 ± 0.84^{b}	68.69 ± 1.76^{b}
Ash (g/100 g DM)	1.62 ± 0.01^{a}	1.74 ± 0.03^{a}	1.95 ± 0.02^{b}
Proteins (g/100 g DM)	5.74 ± 0.09^{c}	4.88 ± 0.08^{b}	4.47 ± 0.05^{a}
Lipids (g/100 g DM)	7.82 ± 1.40^{b}	5.76 ± 0.13^{a}	6.56 ± 0.88^{ab}
Fibre (g/100 g DM)	23.18 ± 0.23^{ab}	22.32 ± 0.83^{a}	20.33 ± 0.21^{a}
Alkaloids (mg/100g)	1967.67 ± 115.47^{b}	967.56 ± 57.73^{a}	933.33 ± 53.75^{a}
Saponin (mg/100g)	1421.33 ± 75.04^{b}	1050.01 ± 20.02^{a}	2557.67 ± 105.42^{c}
Polyphenols (mg/100g)	520.67 ± 21.73^{c}	417.57 ± 6.11^{b}	299.33 ± 3.05^{a}
Tannin (mg/100 g)	132.33 ± 3.06^{b}	103.00 ± 5.29^{a}	166.77 ± 4.74^{c}
Flavonoids (mg/100 g)	1024.67 ± 7.51^{b}	1012.01 ± 2.65^{a}	1084.67 ± 13.50^{c}
IC_{50} DPPH (µg/ml)	423.27 ± 34.20^{b}	825.06 ± 12.13^{c}	372.62 ± 15.40^{a}

Values are expressed as means ±SD; the values on the same column with the same superscript letter are not significantly different at P≤.05

Table 3. Faecal lipid content, blood lipid profile, and the atherogenic indices of the different groups of rats

Rats Group	Faecal lipids [%]	Total cholesterol [mg/dL]	Triglycerides [mg/dL]	HDL [mg/dL]	LDL [mg/dL]	Non-HDL [mg/dL]	AC	AIP
Control	17.72 ± 2.73^{a}	189.37 ± 14.06^{bc}	178.17 ± 26.80^{cde}	46.05±5.32^{a}	112.34 ± 10.21^{bcd}	133.32±7.89^{c}	3.11±0.02^{f}	0.59±0.01^{e}
***Bafia* A**	20.38 ± 1.65^{abcd}	190.47 ± 13.75^{bc}	199.43 ± 10.37^{e}	50.07 ±0.08^{abc}	119.26 ± 39.72^{cd}	130.40±3.89^{c}	2.80±0.01^{e}	0.60±0.01^{e}
***Bafia* B**	20.63 ± 1.56^{bcd}	173.80 ± 29.20^{abc}	202.35 ± 4.93^{e}	56.62±5.89^{bc}	132.69 ± 15.50 ^{de}	117.18 ± 4.62^{b}	2.07 ± 0.03^{c}	0.55 ± 0.02^{d}
***Bafia* C**	23.08 ± 1.89^{def}	151.17 ± 27.73^{a}	155.73 ± 34.86^{bcd}	54.37 ± 6.83^{abc}	95.30 ± 6.68^{abcd}	96.80 ± 3.47^{a}	1.78 ± 0.07^{a}	0.46 ± 0.02^{c}
***Penja* A**	18.86 ± 1.59^{ab}	169.29 ± 2.51^{ab}	180.71 ± 14.04^{cde}	54.48 ± 8.98^{abc}	116.31 ± 35.13^{bcd}	114.81 ±13.02^{b}	2.11 ± 0.04^{c}	0.52 ± 0.02^{d}
***Penja* B**	19.75 ± 0,75^{abc}	198.21 ± 3.58^{c}	173.57 ± 6.66^{cde}	59.02 ± 1.71^{c}	163.94 ± 30.30^{e}	139.19 ± 5.40^{c}	2.36 ± 0.02^{d}	0.47 ± 0.02^{c}
***Penja* C**	21.87 ± 1.89^{cde}	151.02 ± 15.58^{a}	188.49 ± 16.14^{de}	54.05 ± 6.89^{abc}	109.07 ± 8.45^{abcd}	96.97 ± 9.78^{a}	1.79 ± 0.19^{ab}	0.54 ± 0.10^{d}
***Widikum* A**	18.30 ± 1.06^{ab}	180.41 ± 15.25^{bc}	144.54 ± 23.85^{abc}	43.40 ± 10.52^{ab}	76.04 ± 15.19^{ab}	112.01 ±10.93^{b}	2.31 ± 0.09^{d}	0.48 ± 0.09
***Widikum* B**	25.46 ± 0.21^{f}	193.51 ± 7.85^{bc}	111.92 ± 4.31^{a}	59.59 ± 0.76^{c}	68.76 ± 33.13^{a}	103.92 ± 8.51^{ab}	1.74 ± 0.01^{a}	0.27 ± 0.05^{a}
***Widikum* C**	24.00 ± 1.70^{ef}	192.70 ± 8.0^{bc}4	122.72 ± 6.39^{ab}	59.38 ± 1.13^{c}	79.36 ± 18.03^{abc}	113.32 ± 6.38^{b}	1.91 ± 0.04^{b}	0.32 ± 0.08^{b}

A= 5g/kg; B = 10 g/kg; C = 15 g/kg; AC= atherogenic coefficient; AIP= Atherogenic Index of Plasma; Values are expressed as means ±SD; the values on the same column with the same superscripts letter are not significantly different at p≤0.05.

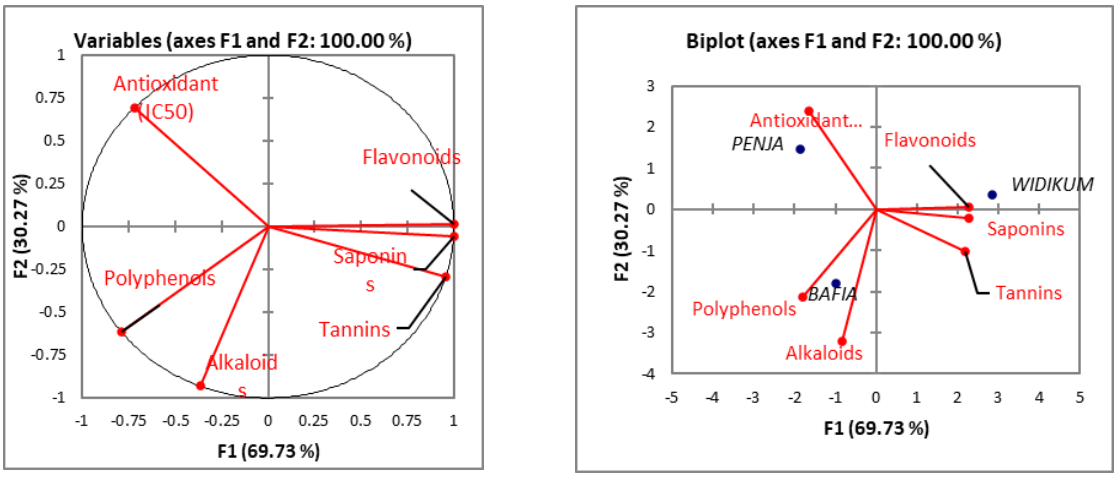

Fig. 1. Distribution of different samples according to their phytochemical content and antioxidant activity

The polyphenols and tannins content obtained in this study is lower than those reported by Adesuyi et al. [33] in *G. kola* with 342 mg/100 g DM and 147 mg/100 g DM of polyphenols and tannin respectively [31].

The flavonoid content ranges from 1012.01 mg/100 g DM (*Penja*) to 1084.67 mg/100 g DM (*Widikum*) and is lower than the values reported by Adesuyi et al. [33] in bitter kola seed (2041 mg/100 g DM) but, higher than those reported by Onyekwelu et al. [29] in the same seeds (790 g/100 g DM). Globally, bitter kola powder analysed is a good source of phytochemicals that can be used to prevent and manage many non-communicable diseases. The *Widikum* variety has the highest content of the phytochemicals analysed, except for total polyphenols and alkaloids.

The antioxidant activities of dry powdered seeds of bitter kola from the 3 different cultivars were evaluated using IC_{50} with gallic acid as a control and the results are presented in Table 2 and Fig. 1. DPPH assay is widely used to determine the free radical-scavenging activity of various extracts or pure compounds. A high EC_{50} indicates the low activity of the sample and vice versa. The EC_{50} of the bitter kola powder varies significantly with the cultivar from 372.62 µg/mL (*Widikum*) to 825.06 µg/mL (*Penja*). Globally, the antioxidant activity of the samples is 10 to 20 times lower than gallic acid used as control, with an EC_{50} of 40.25 µg/mL. The antioxidant activity (DPPH IC_{50}) of the different samples is correlated with saponins (r=-0.76), flavonoids (r=-0.71), and tannins (r=-0.89) contents and is not significantly affected by alkaloids content of bitter kola (r=-0.38). Amongst the different samples, the *Widikum* cultivar is characterised by the highest saponins, flavonoids, and tannins contents and the highest antioxidant activity (fig. 1). Indeed, there is a positive correlation between flavonoids and tannins contents (r=0.95), this could justify the highest antioxidant activity of the *Widikum* cultivar. Flavonoids and tannins are one of the most active dietary antioxidants of the family of polyphenols due to the hydroxyl groups found in their chemical structure. Polyphenolic compounds express their antioxidant activity through their redox properties that allow them to act as reducing agents, hydrogen donors, and single oxygen quenchers [34].

3.2 Effect of Bitter Kola Consumption on Body Weight, Organ Weight, Blood Lipid Profile of Wistar Rats, and Atherogenic Indices

3.2.1 Effect of bitter kola consumption on rats' body and organs weight

The variation in body weight during 3 weeks of rats feeding with feed containing dry seed powders of the 3 cultivars of bitter kola in a high-fat diet at 0, 5, 10, and 15 g per kg of feed was evaluated and the results are presented in fig. 2. The highest weight gain was recorded with the animals in the control group, 22.36% of body weight increase and 23.21±4.22 g of net weight gain (Table 2), indicating that the high-fat diet formulated is efficient to induce obesity.

Globally, the animal weight in the test groups varies in 2 phases and is affected by the cultivar and the proportion of bitter kola in the feed.

During the first 4 days, there was a slight drop in animal weight in the test groups, probably due to the modification of the feed taste by the incorporation of bitter kola. Indeed, these nuts have a bitter and astringent taste and thus can impair the appetite when incorporated into food. In the second phase from the fifth day, there is an increase in body weight varying with the cultivar and the powder incorporation rate in the feed. However, during the test period, the feed consumption did not vary significantly between the different groups (Table 3), thus the weight variation is not due to the modification of taste with the incorporation of bitter kola powder but could be due to the caloric value and the phytochemical contents of the different feed formulated.

Globally, the rats consuming the feed with the *Widikum* cultivar had a lower rate of weight gain during the study. This could be due to its high saponin (2557.67 mg/100 g DM) and flavonoid (1084.67 mg/100 g DM) contents when compared to the other cultivars. The effect of the bitter kola on the prevention of weight gain is dose-dependent. The most effective dose in weight gain inhibition was 15 g of bitter kola powder per kilogram of feed, followed by 10 g of bitter kola powder per kilogram of feed with the lowest being the 5 g of bitter kola powder per kilogram of feed.

The prevention of obesity over time by consuming bitter kola powder indicates that the anti-obesity effect of bitter kola powder could be due to the modification of lipid metabolism, inhibiting excess fat accumulation. Indeed, flavonoids present in bitter kola can modulate several cell-signaling pathways to fat deposition. In addition, alkaloids have been reported to increase energy expenditure and inhibit both adipocyte differentiation and pancreatic lipase [35,36]. Moreover, several studies have reported that the reduction in weight gain is associated with the consumption of food rich in phenolic compounds like bitter kola through several mechanisms.

Polyphenols could inhibit pancreatic lipase activity and lipid absorption [37], form complexes with cholesterol and bile acids and cause their excretion in faeces [38], reduce HMG-CoA reductase activity, inhibit the differentiation of the pre-adipocytes tissues to adipose tissues [39]. The anti-obesity of the bitter kola could be due to hydroxyl citric acid (HCA) present in *G. kola* which is a potent and competent inhibitor of adenosine triphosphate (ATP) citrate lyase, a key enzyme in the synthesis of fatty acids cholesterol and triacylglycerols [40].

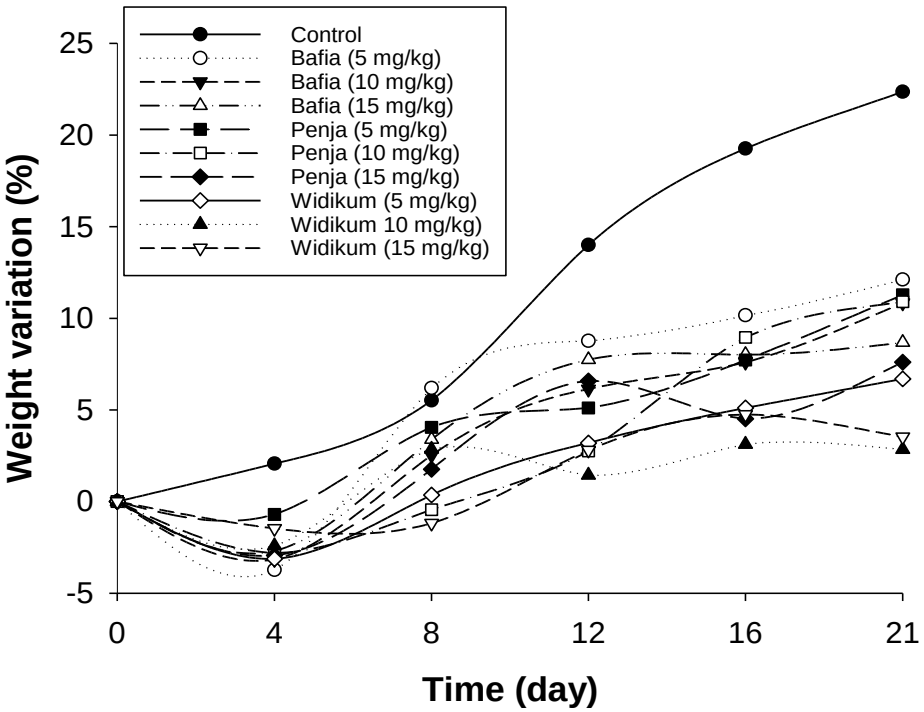

Fig. 2. Effect of variety and dose of bitter kola consumed on rats' body weight

Table 4. Rats' feed intake and weight gain at the end of the study period

Animals groups	Feed intake (g/d)	Weight gain (g)
Control	23.21±4.22[a]	44.72±4.66[d]
***Bafia* A**	24.35±2.64[a]	24.23 ±4,73 [abc]
***Bafia* B**	21.62±3.48[a]	25.69±3.16[bc]
***Bafia* C**	23.01±3.47[a]	21.38±4.70[ab]
***Penja* A**	21.21±2.07[a]	27.58±2.31[c]
***Penja* B**	22.95±4.58[a]	23.82 ±3,81 [abc]
***Penja* C**	21.23±2.96[a]	24.21 ±4,01 [abc]
***Widikum* A**	22.82±2.95[a]	24.38 ±2,75 [abc]
***Widikum* B**	20.48±2.25[a]	19.70±2.56[a]
***Widikum* C**	21.82±3.75[a]	20.05±2.06[ab]

A= 5g/kg; B = 10 g/kg; C = 15 g/kg; Values are expressed as means ±SD; the values on the same column with the same superscripts letter are not significantly different at p≤0.05

HCA also regulates the level of serotonin which has been associated with satiety, increased oxidation of fat, and decreased gluconeogenesis [40], thus, reducing body fat accumulation. The anti-obesity effect of bitter kola may also be attributed to the presence of dietary fibres which act like a physiologic obstacle to lower energy intake by displacement of other nutrients in the diet, providing satiety; and inhibiting food absorption in the small intestine [41]. This result is similar to the results reported by Kuate et al. [42] with a reduction in body weight in obese patients [-11.15% of initial weight] after treatment for 8 weeks with 200 mg/kg of *D. glomerata*.

3.2.2 Effect of bitter kola powder consumption on rat's faecal lipids

The effect of bitter kola consumption on the faecal lipids of the different groups of rats, the blood lipid profile, and the atherogenic index was evaluated and the results are presented in Table 3.

The faecal lipid measures the amount of fat excreted in the faeces and helps to determine the proportion of fat that the body does not absorb after feed ingestion. The faecal lipid excretion varies with the cultivar and the proportion of bitter kola powder in the animal feed. The rats consuming the feed with the *Widikum* cultivar induced the highest fat excretion in their faeces.

There was a positive correlation (r=0.62) between faecal lipids and the incorporation rate of the bitter kola powder in rats' feed, and a negative correlation (r=-74) between the lipid content in the faeces and the weight gain. The control group had the lowest faecal lipid (17.72%) while the rats consuming the feed containing 15 g/kg of powder of bitter kola from Widikum had the highest faecal lipid content (24.00%). This could be due to the bitter kola phytochemicals activity mainly polyphenols that could inhibit pancreatic lipase and facilitate the excretion of triglycerides [35,36], also form complexes with cholesterol and bile acids and cause their excretion in faeces [38]. Indeed, Oishi et al. [43] have reported the anti-obesity potential of a saponin fraction from *Momordica charantia* due to its inhibitory effect on pancreatic lipase in mice. The increase in faecal lipids with the consumption of bitter kola powder could also be due to the reduction of the expression of hypothalamic neuropeptide Y and serum leptin [44] by the bitter kola powder phytochemicals. Similar results were reported by Kim et al. [44] on the anti-obesity effect of the crude saponin fraction of Korean red ginseng (*Panax ginseng*) on rats fed with a high-fat diet.

3.2.3 Effect of bitter kola consumption on Wistar rats' blood lipid profile and atherogenic indices

Hyperlipidaemia is one of the major risk factors for some cardiovascular diseases such as atherosclerosis, and cholesterol is the major lipid constituent of atherosclerotic plaque [45]. Epidemiological and clinical studies show that serum level of HDL is inversely related to the incidence of coronary artery disease. The higher the serum HDL cholesterol concentration, the lower the incidence of coronary artery disease. The effect of the different cultivars and different doses of bitter kola on blood lipid profile and atherogenic indices is presented in Table 3. The serum lipid profile of the different groups of rats varies significantly with the bitter kola species and the dose in the rat feed. Globally, the different test groups have lower serum total cholesterol, triglycerides, and HDL for the doses of 10 g and 15 g of bitter kola powder per kilogram of feed respectively. Amongst the different samples, the *Widikum* cultivar was the

most active while the *Penja* cultivar was the less active. The low activity of the *Penja* cultivar could be because Penja is one of the agroecological areas with modern agricultural practices where the use of fertiliser is common and may reduce the production of phytochemicals, in opposition to other localities where the bitter kola is produced more naturally.

To evaluate the potential of the bitter kola powder to prevent cardiovascular diseases on a high-fat diet, the atherogenic index of plasma (AIP) and atherogenic coefficients (AC) were calculated and the results are presented in Table 3. AIP is significantly associated with CV risks [46]. From the results obtained, the control group fed with a high-fat diet without bitter kola powder has the highest AIP value (0.59-0.60). The AIP of the different test groups of rats varies with bitter kola cultivars and the dose in rats' feeds. The lowest AIP reduction was obtained with the *Penja* cultivar while the highest reduction was obtained with the *Widikum* cultivar. Moreover, the AIP values are negatively correlated with the bitter kola proportion in feed, the rats consuming the feed with 10 g of powder of *Widikum* variety per kilogram of feed had the lowest AIP (0.27). The AIP has been shown to correlate with the size and composition of lipoproteins, people with high AIP have a high risk for coronary artery disease. AIP is composed of triglycerides and high-density lipoprotein cholesterol and it is considered as a predictive marker for plasma atherogenicity and cardiometabolic health with high sensitivity. The reduction of AIP is correlated to the reduction of some lipoprotein subclasses, mainly the amounts of LDL, improving the blood lipid profile and reducing the susceptibility of lipoprotein to oxidation [46].

AC is a measure of cholesterol in LDL, VLDL, and IDL fractions concerning the cholesterol in HDL [28]. AC reflects the atherogenic potential of the entire spectrum of lipoprotein fractions and hence indicates the CV risk [47]. The results obtained indicate that the control group has the highest AC (3.11), the consumption of the bitter kola powder by the rats in the different test groups reduces the AC and thus the risk of cardiovascular diseases. The most effective dose was 10 g/kg of feed with the *Widikum* cultivar (1.74).

Non-HDL cholesterol serves as an index of cardiovascular risk in some groups of population like diabetic patients in whom LDL may not be elevated. From the results in Table 3, the consumption of bitter kola powder affects significantly the non-HDL cholesterol level in the serum, with the effect varying with the variety and does. The control group has the highest non-HDL cholesterol level (133.32 mg/dL) while the group consuming 15 g of *Bafia* cultivar powder per kilogram of feed has the lowest non-HDL cholesterol level (96.80 mg/dL). From these results, bitter kola powder has anti-obesity potential and can protect against coronary artery diseases. The anti-obesity activity of the bitter kola powder could be due to the cumulative effect of phytochemicals present in bitter kola nuts, such as xanthones, polyphenols, flavonoids, and benzophenone present in the fruit of some *Garcinia* with hypolipidemic effects [48]. Indeed, flavonoids from *G. cambogia* fruit reduced serum lipid levels in rats fed with a cholesterol-rich diet by decreasing lipogenesis and enhancing the degradation of lipids [49]. Moreover, *Garcinia* species contains hydroxy citric acid (HCA), a competitive inhibitor of the citrate cleavage enzyme, ATP-citrate lyase, and this inhibitory effect reduces the rate of lipogenesis, resulting in a hypolipidemic effect [50].

Tables should be explanatory enough to be understandable without any text reference. Double spacing should be maintained throughout the table, including table headings and footnotes. Table headings should be placed above the table. Footnotes should be placed below the table with superscript lowercase letters. Sample table format is given below.

3.2.4 Effect of bitter kola consumption on the organ indices

The effect of bitter kola powder consumption on the liver, kidney, lungs, and spleen indices was evaluated and the results are presented in Table 5. For the different test groups of rats, there was no significant variation in organ indices. However, the kidney, liver, and heart indices of the rats of the control group are significantly higher compared to the test groups. This could be due to the fat accumulation in rats' organs in the control prevented in the test groups by the consumption of bitter kola powder. Moreover, the constant and lower values of the organ indices in the test groups indicate that the doses of bitter kola powder used in this study are not affecting negatively the different organs and thus are not toxic as the relative variation in organ indices after ingestion of a substance indicates its toxicity [50].

Table 5. Organ indices (in %) of rats at the end of the test period

Groups	kidney	Liver	Heart	Spleen
Control	0.89±0.25^{b}	5.67±0.20^{b}	0.36±0.08^{b}	0.34±0.07^{ab}
***Bafia* A**	0.80±0.06^{ab}	3.38±0.29^{a}	0.32±0.10^{ab}	0.36±0.07^{ab}
***Bafia* B**	0.62±0.27^{a}	3.87±0.62^{a}	0.28±0.09^{a}	0.26±0.19^{ab}
***Bafia* C**	0.71±0.09 ^{ab}	3.70±0.28^{a}	0.28±0.06^{ab}	0.30±0.03^{ab}
***Penja* A**	0.78±0.06 ^{ab}	4.15±0.15^{ab}	0.33± 0.03^{ab}	0.38±0.05^{b}
***Penja* B**	0.74±0.11 ^{ab}	4.10±0.29^{ab}	0.36± 0.05^{b}	0.37±0.10^{b}
***Penja* C**	0.82±0.07 ^{ab}	3.44±0.10^{a}	0.32± 0.03^{ab}	0.31±0.08^{ab}
***Widikum* A**	0.77±0.06 ^{ab}	3.65±0.38^{a}	0.37±0.04^{ab}	0.25±0.04^{a}
***Widikum* B**	0.73±0.10^{ab}	3.49±0.28^{a}	0.29±0.10^{b}	0.35±0.05^{ab}
***Widikum* C**	0.77±0.13^{ab}	3.88±1.11^{ab}	0.32±0.08^{ab}	0.34±0.08^{ab}

A= 5g/kg; B = 10 g/kg; C = 15 g/kg; Values are expressed as means ±SD; the values on the same row with the same superscripts letter are not significantly different at p≤0.05

4. CONCLUSION

This study was aimed at determining the effect of bitter kola consumption in a high-fat diet on lipid profile, atherogenic indices, and body weight. Results obtained revealed that the bitter kola powder composition varies with the cultivar and the *Widikum* has the highest content of most of the phytochemicals and the highest antioxidant activity. The incorporation of the bitter kola powder up to 15 g/kg of feed did not affect the feed consumption rate. The consumption of bitter kola powder in a high-fat diet reduces weight gain and improves the serum lipid profile of the rats. The consumption of bitter kola powder also reduces the atherogenic index of plasma and the atherogenic coefficient, the best reduction was obtained with the *Widikum* cultivar at the incorporation rates of 10 g and 15 g of powder per kilogram of feed. Thus, bitter kola powder has good anti-obesity and hypolipidemic activities and can be used to prevent coronary artery disease and its complications.

ETHICAL APPROVAL

The authorization to conduct the research and ethical approval were granted by the College of Technology Scientific Committee Board, The University of Bamenda (ref N°02/23/UBa/COLTECH/D/NFBT-HOD of 24/03/2023).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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