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Effects of Mucuna milk (*Mucuna pruriens L.*) on body weight and serum biochemistry in rats fed hyperlipidaemic diet

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Abstract

Background Hyperlipidemia and oxidative stress is recognized as risk factor for several diseases such as COVID-19. The aim of this study was to evaluate the effect of oral administration of *Mucuna* milks on body weight gain, blood lipid levels and redox status in rat model fed high fat diet.

Methods *Mucuna* milks were produced from two varieties of *Mucuna* seeds. Three controls (I, II, III) made of normal rats fed with standard diet, rats fed with high fat diet and rats fed with high fat diet submitted to oral administration of atorvastatin (10 mg/kg/day). In addition, four test groups (IV, V, VI, VII) made with rats fed high fat diet and received oral administration of 20 mL of vegetable milk per day (10 mL at morning and 10 mL at afternoon).

Results Results confirmed that rats on the high fat diet only showed an increasing of about 224% of their initial body weight, abdominal fat and a significant ($p < 0.05$) increases of lipid peroxidation (MDA) in liver and heart after five weeks. However, oral administration of *Mucuna* milk inhibit weight gain by about 66% and abdominal fat (54.53–55.60%). The reduction of LDL, VLDL, Triglycerides and Total cholesterol was remarkable in groups of rat treated with vegetable milk, about 67% reduction for dehulled *Mucuna* milks (DCM, DVM) and 69% reduction for whole *Mucuna* milks (WCM, WVM). Hyperlipidemic group rats had higher ASAT (134.17 UI/L) and ALAT (101.72 UI/L) levels, but *Mucuna* milks improved the ASAT and ALAT levels in rats. The reduction of MDA (70 − 50%) was related to phenolic content of *Mucuna* milks. significant and negative correlations was observed between catalase and MDA (r= -0.86; $p = 0.05$); MDA and SOD ($r = -0.60$; $p = 0.05$).

Conclusion This study suggests that treatment with *Mucuna* milks have anti-hyperlipidemia properties and increased the activity of antioxidant enzymes.

Keywords *Mucuna* milk, High fat diet, Serum biochemistry, Antioxidant properties

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Background

The changes in lifestyle resulting from industrialization impact significantly on the health of the populations. In other words, the modernization of societies has driven populations to consume foods that are richer in saturated fats and refined sugars and lower in fiber. Countries in Central Africa have also been influenced by this trend and they are transiting more and more towards a Western lifestyle. As a result, their traditional foods mainly composed of beans, roots, cereals, tubers and vegetables have been replaced by fatty foods, high sugar snacks and drinks too rich in calories [1].

These shifts in eating habits, together with changes in physical activity behaviors, are potential causes of hyperlipidemia and obesity [2]. A number of studies have shown that hyperlipidemia is the main risk factor for atherosclerosis, which is the primary cause of mortality and disability in many countries [3]. Moreover, Vuorio et al. [4] reported hyperlipidemic patients are more exposed to COVID-19 complications during the acute phase of the infection over a long period of time.

In recent time, the development of lipid lowering drug or formulation from natural source has gained importance. In this respect, the study of food for antioxidant and hypolipidemic activities may give new pharmacological approach in the treatment of hyperlipidemia [5]. Thus, it has been shown that pulses extract and leguminous isolated proteins reduce elevated serum cholesterol and triglycerides [6–8]. In addition, studies have shown that vegetable milk produced with common leguminous seeds, such as soya milk and peanut milk, possess hypolipidemic and antioxidant properties that may be useful in the reducing the risk of cardiovascular diseases [9]; [10].

In our previous study, we have studied the optimal conditions for *Mucuna* milk production [11]; [12]; [13], the chemical composition and proteins digestibility of *Mucuna* milk [14] have also been evaluated in vivo. The result obtained showed that this vegetable milk is not only a good source of proteins, but its consumption lower serum cholesterol and triglycerides in young normal rats fed diets formulated with it as a proteins source [14]. In fact, *Mucuna pruriens* belongs to the family of fabaceae, the seeds are used as a soup thickener by rural population in Far-North region of Cameroon and also eaten in south-eastern Nigeria, Indian tribal sects, Mundari and Dravidian groups [15]. Nutritional quality of Mucuna seeds is comparable to soya beans as it contains similar proportions of proteins, lipids, minerals, and other nutrients. The purpose of this study was to test whether *Mucuna* milk can also influence body weight gain, blood lipid levels and redox status in rat model fed high fat diet.

Materials and methods

Sampling and production of *Mucuna* milk

The two varieties of *Mucuna pruriens* seeds (var. Cochinchinensis and var. Veracruz mottle) used for this study were obtained from the International Institute of Tropical Agriculture (IITA) of Yaoundé, Cameroon. The samples were assigned a voucher specimen number PQG/I/2020/2021/015 after identification. *Mucuna* bean flours and vegetable milk samples were produced as previously described by Mang et al. [12, 13] respectively. In this respect 8 g of *Mucuna* flour was blended with 100 mL of distilled water. The slurry was stirred at 3500 rpm using an electric stirrer (TECHNICON stirrer motor, England) during 60 min, under extraction temperature of 60 °C maintained with controlled temperature water bath. After incubation, the sample was centrifuged at 1500 g for 15 min at 20 °C using refrigerated ultracentrifuge. The supernatant was collected and the residues were re-extracted in the same conditions. The collected supernatants were combined and packaged in 100 mL volumetric glass vessels and stored at 4 °C in the refrigerator for analysis within a maximum of 4 h. The vegetables milks resulting from *Mucuna Veracruz* flours were coded WVM (Whole Veracruz milk), DVM (Dehulled Veracruz milk), and *Mucuna Cochinchinensis* flours were coded WCM (Whole Cochinchinensis milk), DCM (Dehulled Cochinchinensis milk).

Animal experiments and biological assay

Experimental design

The experimental procedures described below were approved by the institutional animal ethical committee of Higher Technical Teachers' Training College of Ebolowa, University of Yaounde I. Fourthy two healthy male Wistar rats (weighing 130–145 g, three months' rats) were divided into 7 groups each containing 6 rats. The animals were procured from the animal house of National School of Agro-Industrial Sciences, Ngaoundéré University, Cameroon and were kept in cages, 1 per cage, with relative humidity (55%) in a 12 h light/dark cycle at 25±2 °C. Before experimentation, they were given access to water and a standard diet ad libitum.

Then, as illustrated in Table 1, Group I was made of normal control rats fed with standard diet; Group II consisted of rats receiving high fat diet only; Group III was composed of rats fed with high fat diet and treated with standard drug, Atorvastatin (10 mg/kg per day) orally for 4 weeks; Group IV, V, VI and VII were tests groups made with rats fed with high fat diet and received oral administration of 20 mL of vegetable milk per day (10 mL at morning and 10 mL at afternoon). The animals of groups IV, V, VI and VII were respectively treated with dehulled Cochinchinensis milk (DCM), whole Cochinchinensis milk (WCM), dehulled Veracruz milk (DVM) and whole

Table 1 Description of treatment administrated to experimental rats

Groups	Treatments
Group I	Fed with normal diet (Normal control group)
Group II	Fed with High fat diet (Hyperlipidemic control group)
Group III	Fed with High fat diet + Atorvastatin (10 mg/kg/day) (Atorvastatin standard control group)
Group IV	Fed with High fat diet + received oral administration of 20 mL of dehulled Cochinchinensis milk per day (10 mL at morning and 10 mL at afternoon) (DCM group)
Group V	Fed with High fat diet + received oral administration of 20 mL of whole Cochinchinensis milk per day (10 mL at morning and 10 mL at afternoon) (WCM group)
Group VI	Fed with High fat diet + received oral administration of 20 mL of dehulled Vera cruz milk per day (10 mL at morning and 10 mL at afternoon) (DVM group)
Group VII	Fed with High fat diet + received oral administration of 20 mL of whole Vera cruz milk per day (10 mL at morning and 10 mL at afternoon) (WVM group)

Veracruz milk (WVM). Rats received water and their experimental diets ad libitum. The compositions of the standard and high fat diet were as follow:

Standard Diet (SD): Cassava starch 60%, Sucrose 5%, Casein 10%, Tournesol oil 10%, salt mixture with starch 5%, Cellulose 5%, Vitamin mixture 4% and mineral mixture 1% [6].

High Fat Died (HFD): Cassava starch 25%, Sucrose 5%, Casein 10%, Cholesterol 10%, Tournesol oil 10%, salt mixture with starch 5%, coconut oil 25%, cellulose 5%, Vitamin mixture 4% and mineral mixture 1% [16].

Measurement of body weight, food intake and collection of faeces

Individual body weight of rats was measured weekly using a weighing balance. The percentage weight gain (%) was calculated as: (body weight on specific week (g) − initial body weight)/initial body weight ×100. Feed intake and feed waste were recorded every day (over 24 h) based on the weight of leftover feed out of 100 g given. Fecal samples were recorded at the start (week 1) at middle (week 2) and at the final stage (week 4) of the study in order to evaluate the mean of fecal lipids using soxhlet method [17].

Blood sampling and biochemical analysis

After 12 h of last administration, overnight-fasted animals were anaesthetized by inhalation of isoflurane impregnated on a cotton wool and sacrificed. Abdominal fat was also carefully dissected and weighed. The blood was collected from heart puncture into a vacuum tube and centrifuged at 3000 rpm for 10 min and clear serum was aspirated, stored frozen and then used for desired analysis. Analysis of serum for total cholesterol (TC) [18], triglycerides [19], and high density lipoprotein cholesterol (HDL-c) [20], glucose, creatinine, aspartate aminotransferase (AST) [21] and alanine aminotransferase (ALT) [21] was performed by using commercial kits and following standard procedures outlined by the producer, Randox Laboratories, UK. Serum low density lipoprotein cholesterol (LDL-c) concentration and very low density lipoprotein cholesterol (VLDL-c) were determined using Friedwald formula, where LDL-c = TC − (HDL-c + VLDL-c) and VLDL-c = TG/5 [22].

In vivo analysis of the antioxidant activity

For evaluation of in vivo antioxidant activity, liver and heart tissues were separately minced and homogenized (10% w/v) in 0.1 M phosphate buffer (pH 7.0) and centrifuged at 5000 × g for 10 min and the resulting supernatant was used.

Lipid peroxidation in tissue homogenate was estimated by the colorimetric quantification of Malondialdehyde (MDA) [23]. In the procedure 0.1 mL of homogenate was treated with 2 mL of (1:1:1 ratio) TBA-TCA-HCl reagent (TBA 0.37%, 0.25 N HCl and 15% TCA) and placed in water bath for 15 min and cooled. The absorbance of clear supernatant was measured against reference blank at 535 nm. The lipid peroxidation was calculated on the basis of the molar extinction coefficient of MDA and expressed as nmoles MDA/mg protein.

The catalase activity was determined by adopting the method of [24]. The reaction mixture contained 1.0 mL of 0.01 M pH 7.0 phosphate buffer, 0.1 mL of tissue homogenate and 0.4 mL of 2 M H_2O_2 in a total volume of 1.5 mL. The reaction was stopped by the addition of 2.0 mL of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid mixed in 1:3 ratios). Then the absorbance was measured at 620 nm and the catalase activity expressed as μmoles of H_2O_2 consumed/min/mg protein.

The activity of Superoxide Dismutase (SOD) activity was assayed by the method of [25]. 0.5 mL of tissue homogenate was mixed with 1 mL of distilled water, then 2.5 mL of ethanol and 1.5 mL of chloroform were added, shaken for 1 min at 4 °C and the tube centrifuged to collect the supernatant. The assay mixture containing 1.2 mL of sodium pyrophosphate buffer (0.025 M, pH 8.3), 0.1 mL of 186 μM PMS, 0.3 mL of 30 μM NBT, 0.2 mL of 780 μM NADH, appropriately diluted enzyme preparation and water in a total volume of 3 mL. Reaction was started by the addition of NADH. After incubation at 30 °C for 90 s the reaction was stopped by the addition of 1 mL glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4 mL of n-butanol. The intensity of the chromogen in the butanol layer was measured at 560 nm against butanol blank. Assay mixture devoid of enzyme served as control. One unit of the enzyme activity is defined as the enzyme reaction, which gave 50%

inhibition of NBT reduction in one minute under the assay conditions.

Statistical analysis

The data reported in the tables and figures were carried out in triplicate or more replicate determinations. All data were expressed as mean ± standard deviation and were statistically analyzed using one way analysis of variance (ANOVA). When statistical differences were found, the Duncan's Multiple Range Test was applied in order to classify samples at the significant level of 5%. Stat graphics Program (Statically Graphics Educational, version 6.0 1992 Manugistics, Inc. and Statistical Graphics Corp., USA) was used for the statistical analysis.

Results

Effect of *Mucuna pruriens* milk on body weight, food intake and faecal fat excretion

As illustrated in Fig. 1, rats on the high fat diet only showed an increasing of about 224% of their initial body weight after five weeks, while oral administration of vegetable milk reduced weight gain. Rats receiving whole *Mucuna* milk together with the HFD, showed no significant difference ($p<0.05$) in weight gain as compared to rats receiving atorvastatin. In addition, compared to rats receiving HFD, it is noted that these vegetable inhibit weight gain by about 66%, whole Veracruz milk (WVM) seems to be the most effective because it inhibits weight gain by about 70%.

In terms of food intake (Table 2), rats fed with normal diet consumed more in terms of quantity (22.60 g) as compared to the HFD group only (16.42 g) but have lesser weight gain, thus confirming the higher calorie intake in the HFD group. Oral administration of *Mucuna* milk enhance significantly ($p<0.05$) food intake in HFD group. Thus, *Mucuna* milk induces a significant ($p<0.05$) increase in food intake, around 5% for dehulled *Mucuna* milk (DCM and DVM) and 20.60% for whole *Mucuna* milk (WCM and WVM). The difference in weight gain among the different groups is clearly demonstrated by the abdominal fat, where rats on the HFD and whole Mucuna milk (WCM and WVM) had significantly ($p<0.05$) reduced percentage of abdominal fat (54.53–55.60%) as compared to rats on the HFD only.

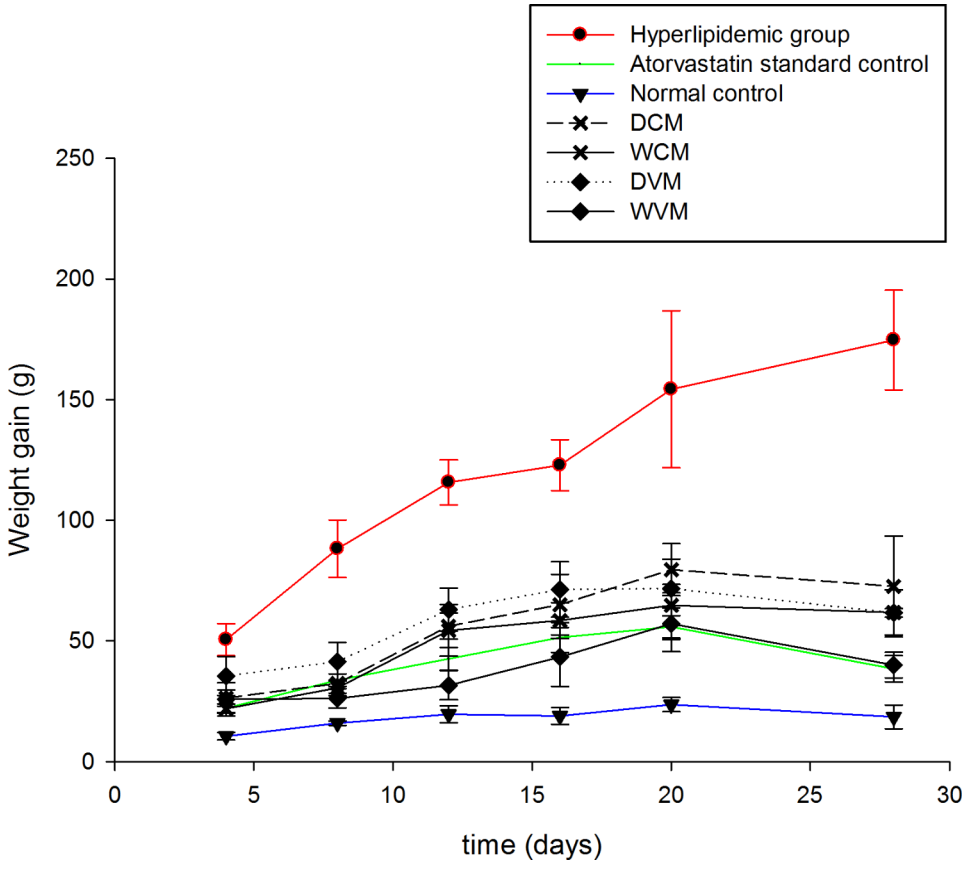

Fig. 1 Effect of *Mucuna* milk oral administration on weight gain of rats fed with high-fat diet
Results are the means ± SD (n = 6) of six animals. DCM: dehulled Cochinchinensis milk; WCM: whole Cochinchinensis milk; DVM: Dehulled Veracruz milk; WVM: whole Veracruz milk

Table 2 Effect of *Mucuna pruriens* milk oral administration on food intake, fecal lipids and abdominal fat of rats fed with high fat diet

Groups	Parameters		
	Food intake (g/day/rat)	Faecal fat content (%)	Abdominal fat (g)
Normal control	22.60 ± 0.80 [d]	0.97 ± 0.02 [a]	2.11 ± 0.12[a]
Hyperlipidemic group	16.42 ± 0.65[a]	1.31 ± 0.05 [b]	24.17 ± 4.02 [d]
Atorvastatin standard control	19.38 ± 0.65 [c]	4.12 ± 1.04 [c]	11.96 ± 1.39 [b]
DCM	17.13 ± 0.68 [b]	5.03 ± 1.16 [c]	13.05 ± 1.39 [b]
WCM	19.46 ± 0.62 [c]	5.66 ± 1.10 [c]	10.99 ± 1.68 [b]
DVM	17.08 ± 0.64 [b]	4.98 ± 1.40 [c]	15.01 ± 1.99 [c]
WVM	19.80 ± 0.60 [c]	6.06 ± 1.31 [c]	10.73 ± 1.90 [b]

Means ± SD (n = 6) followed by different letters in the same line are significantly different ($p < 0.05$) as determined by Duncan's multiple range test. DCM: dehulled Cochinchinensis milk group; WCM: whole Cochinchinensis milk group; DVM: Dehulled Veracruz milk group; WVM: whole Veracruz milk group

Effect of *Mucuna pruriens* milk on serum biochemistry

As illustrated in Table 3, oral administration of *Mucuna* milk positively influenced ($p < 0.05$) the lipid profiles in the treated group receiving the high fat diet. Moreover, dehulling of Mucuna seeds appears to significantly ($p < 0.05$) influence the effectiveness of these vegetable milks. Total cholesterol reduction is remarkable in groups of rat treated with vegetable milk, about 67% reduction for dehulled *Mucuna* milks (DCM, DVM) and 69% reduction for whole *Mucuna* milks (WCM, WVM). A reduction of LDL, VLDL and Triglycerides level was also recorded in treated groups. It's observable in Table 4 that the HFD only group had significant ($p < 0.05$) higher blood glucose levels at the end of experimentation (278.11 ± 14.49 mg/dL) as compared to rats receiving the normal diet (81.51 ± 1.35 mg/dL). Administration of *Mucuna* milk improved blood glucose, dependently of seeds dehulling with rats receiving whole *Mucuna* milks (WCM, WVM) having the most improved profile (84.25–84.15 mg/dL), followed by rats receiving dehulled *Mucuna* milk (DCM, DVM) (95.51–93.75 mg/dL). The creatinine levels among the various groups were not significantly different, in both rats fed the HFD (0.88 mg/dL) or the normal diet (0.89 mg/dL). In the current study, three markers of liver function were measured (ASAT, ALAT and Total Protein) (Table 5). Significant changes ($p < 0.05$) were observed in the ASAT and ALAT levels of rats. Hyperlipidemic group rats had significantly ($p < 0.05$) higher ASAT (134.17 UI/L) and ALAT (101.72 UI/L) levels than normal rats, with 18.92 and 20.41 UI/L, respectively. Oral administration of different *Mucuna* milks improved the ASAT and ALAT levels in rats fed a high fat diet. Then, this vegetable milk reduced hepatic liver injury, as reflected by decreased levels of ALT and AST. No significant findings were recorded, especially in the treated group, suggesting that *Mucuna* milk was not toxic at the administered dosages (Table 6).

Table 4 Effect of *Mucuna pruriens* milk oral administration on blood glucose of rats fed with high fat diet

Groups	Glucose (mg/dl)	
	Start of experiment	End of experiment
Normal control	82.5 ± 1.16[a]	81.51 ± 1.35[a]
Hyperlipidemic group	83.01 ± 1.36[a]	278.11 ± 14.49[b]
Atorvastatin standard control	83.75 ± 2.21[a]	101.25 ± 6.99[b]
DCM	81.02 ± 2.59[a]	93.75 ± 3.59[b]
WCM	82.25 ± 1.90[a]	84.15 ± 3.86[a]
DVM	83.20 ± 1.96[a]	95.51 ± 2.38[b]
WVM	81.75 ± 2.56[a]	84.25 ± 3.30[a]

Means ± SD (n = 6) followed by different letters in the same line are significantly different ($p < 0.05$) as determined by Duncan's multiple range test. DCM: dehulled Cochinchinensis milk group; WCM: whole Cochinchinensis milk group; DVM: Dehulled Veracruz milk group; WVM: whole Veracruz milk group

Antioxidant potential of *Mucuna* milk in rats fed hyperlipidaemic diet

Figure 2 show that consumption of high fat diet induces a significant ($p < 0.05$) increases of lipid peroxidation (MDA) in liver and heart. Significantly decreased levels ($p < 0.05$) of lipid peroxidation in administration of Mucuna milks in tissues of rats fed high fat diet when compared with normal control rats was observed (Fig. 2). MDA reduction was about 70% for liver and 50% for heart. As illustrated in Figs. 3 and 4, activities of SOD and Catalase, antioxidants were significantly ($p < 0.05$)

Table 3 Effect of *Mucuna pruriens* milk oral administration on Total Cholesterol, Triglycerides, HDL, LDL and VLDL in serum of rats fed with high fat diet

Serum Parameters	Normal control	Hyperlipidemic control	Atorvastatin standard control	var. Cochinchinensis		var. Veracruz	
				DCM	WCM	DVM	WVM
Total Cholesterol (mg/dl)	89.32 ± 12.16[a]	344.57 ± 170.43[c]	120.05 ± 10.14[b]	106.12 ± 11.52[a]	91.75 ± 12.33[a]	97.70 ± 13.10[a]	90.92 ± 11.61[a]
Triglycerides (mg/dl)	81.55 ± 8.93[b]	246.52 ± 6.65[c]	101.45 ± 13.10[b]	105.20 ± 2.41[b]	92.52 ± 15.60[b]	71.82 ± 7.83[b]	58.42 ± 6.15[a]
HDL-C (mg/dl)	39.67 ± 5.01[b]	22.78 ± 5.79[a]	33.61 ± 6.55[b]	33.50 ± 5.21[b]	31.75 ± 5.73[b]	30.42 ± 4.51[b]	32.72 ± 6.47[b]
LDL-C (mg/dl)	33.33 ± 12.88[a]	242.49 ± 167.59[b]	66.14 ± 12.52[a]	51.58 ± 30.62[a]	42.24 ± 8.74[a]	52.91 ± 15.87[a]	46.51 ± 12.21[a]
VLDL-C (mg/dl)	16.31 ± 1.78[c]	49.30 ± 1.33[f]	20.29 ± 1.62[e]	21.04 ± 0.48[e]	18.50 ± 1.12[d]	14.36 ± 1.56[b]	11.68 ± 1.23[a]

Means ± SD (n = 6) followed by different letters in the same line are significantly different ($p < 0.05$) as determined by Duncan's multiple range test. DCM: dehulled Cochinchinensis milk group; WCM: whole Cochinchinensis milk group; DVM: Dehulled Veracruz milk group; WVM: whole Veracruz milk group

Table 5 Effect of *Mucuna pruriens* oral administration on serum creatinine, ALAT, ASAT and total proteins of rats fed with high fat diet

Groups	Parameters			
	Creatinine (mg/dL)	ALAT (UI/L)	ASAT (UI/L)	Total Protein (mg/dL)
Normal control	0.89 ± 0.05^{ab}	20.41 ± 2.40^{a}	18.92 ± 3.27^{a}	7.09 ± 0.59^{c}
Hyperlipidemic group	0.88 ± 0.05^{ab}	101.72 ± 10.29^{d}	134.17 ± 7.08^{d}	3.99 ± 0.44^{a}
Atorvastatin standard control	0.86 ± 0.08^{ab}	29.35 ± 3.39^{b}	28.15 ± 2.90^{b}	5.28 ± 0.33^{b}
DCM	0.87 ± 0.09^{ab}	32.10 ± 1.56^{b}	37.07 ± 3.61^{bc}	6.90 ± 0.70^{c}
WCM	0.83 ± 0.06^{a}	20.45 ± 1.77^{a}	26.45 ± 1.34^{a}	6.56 ± 0.55^{c}
DVM	0.87 ± 0.03^{ab}	37.90 ± 4.13^{c}	39.50 ± 2.59^{c}	6.77 ± 0.51^{c}
WVM	0.88 ± 0.03^{ab}	22.06 ± 3.02^{a}	29.26 ± 1.25^{a}	6.34 ± 0.48^{c}

Means ± SD (n = 6) followed by different letters in the same line are significantly different ($p < 0.05$) as determined by Duncan's multiple range test. DCM: dehulled Cochinchinensis milk group; WCM: whole Cochinchinensis milk group; DVM: Dehulled Veracruz milk group; WVM: whole Veracruz milk group

Table 6 Effect of *Mucuna pruriens* milk oral administration on organ weights of rats fed with high fat diet

Groups	Parameters (g)			
	Liver	Kidney	Heart	Lung
Normal control	9.10 ± 0.80^{a}	2.21 ± 0.18^{a}	1.27 ± 0.12^{a}	1.41 ± 0.12^{a}
Hyperlipidemic group	9.22 ± 0.65^{a}	2.18 ± 0.45^{a}	1.21 ± 0.15^{a}	1.57 ± 0.21^{a}
Atorvastatin standard	9.02 ± 0.65^{a}	2.02 ± 0.71^{a}	1.18 ± 0.21^{a}	1.46 ± 0.19^{a}
DCM	8.93 ± 0.68^{a}	2.13 ± 0.84^{a}	1.23 ± 0.16^{a}	1.35 ± 0.33^{a}
WCM	8.96 ± 0.72^{a}	2.16 ± 0.27^{a}	1.26 ± 0.17^{a}	1.29 ± 0.22^{a}
DVM	9.08 ± 0.74^{a}	2.11 ± 0.32^{a}	1.29 ± 0.30^{a}	1.32 ± 0.19^{a}
WVM	8.80 ± 0.80^{a}	2.15 ± 0.30^{a}	1.25 ± 0.31^{a}	1.43 ± 0.32^{a}

Means ± SD (n = 6) followed by different letters in the same line are significantly different ($p < 0.05$) as determined by Duncan's multiple range test. DCM: dehulled Cochinchinensis milk group; WCM: whole Cochinchinensis milk group; DVM: Dehulled Veracruz milk group; WVM: whole Veracruz milk group

decreased in liver and heart of hyperlipidemia control rats. There is no significant difference on the effect of different milks on Catalase activity (Fig. 3). However, it is noted that, in Fig. 4 vegetable milks produced from undehulled *Mucuna* seeds more increase the activity of SOD in the heart.

Discussion

It is known that, an excess amount of caloric supply may lead to obesity [26]. In this study, results showed that rats fed a high-fat diet for a long period of time suffer from obesity and have excess fat accumulation when compared to normal rats [27]; [28]. However, there was a significant ($p < 0.05$) increase in food intake in the HFD group upon oral administration of plant milk, which indicates that suppression of appetite alone may not be the major anti-obesity mechanism involved in this study. An inhibition of digestive lipases might have been the most likely mechanism for this effect. These findings are quite similar to those reported by many other studies using different plant extracts [29]; [30]. Previous studies have suggested that Mucuna milk may have an anti-hyperlipidemic effect through the inhibition of metabolic and digestive lipases by reducing the intake of calories [31]; [32]. Furthermore, enzyme inhibition was determined by assessing the fecal fat content. Rats fed with Mucuna milk had a higher fecal fat content than normal control rats. This would indicate that lipase was inhibited by Mucuna milk in vivo. This result is consistent with previous findings that showed that natural anti-hyperlipidemic agents such as green tea can increase the energy content of feces in rats [33] and that tea catechins can increase fecal excretion of these energy nutrients by inhibiting digestive enzymes [34].

Apart from increased weight and fat accumulation, hyperlipidemia is associated with other physiological disruptions, which is reflected by changes in the serum biochemistry [32]. In this study, elevated lipidemia was noted in untreated hyperlipidemic rats, but the drop observed in rats treated with Mucuna milk could be attributed to the phenolic compounds of *Mucuna* milk. In fact, phenolic compounds have been reported to positively modulate cholesterol metabolism [35]. Our previous work has shown that Mucuna milk was rich in phenolic compounds, including flavonoids, tannins and Vitamin C [12]; [13]. The cholesterol lowering potential of phenolic compounds was mediated through the inhibition of HMG-CoA reductase and ACAT activity and an increased fecal sterol excretion [36].

The glomerular filtration rate, which is used to measure the overall kidney function, cannot be directly estimated. Therefore, creatinine and urea are often used as indicators of renal function in clinical trials [37]. There was not a significant difference in creatinine levels between rats fed an HFD (0.88 mg/dL) and those fed a normal diet

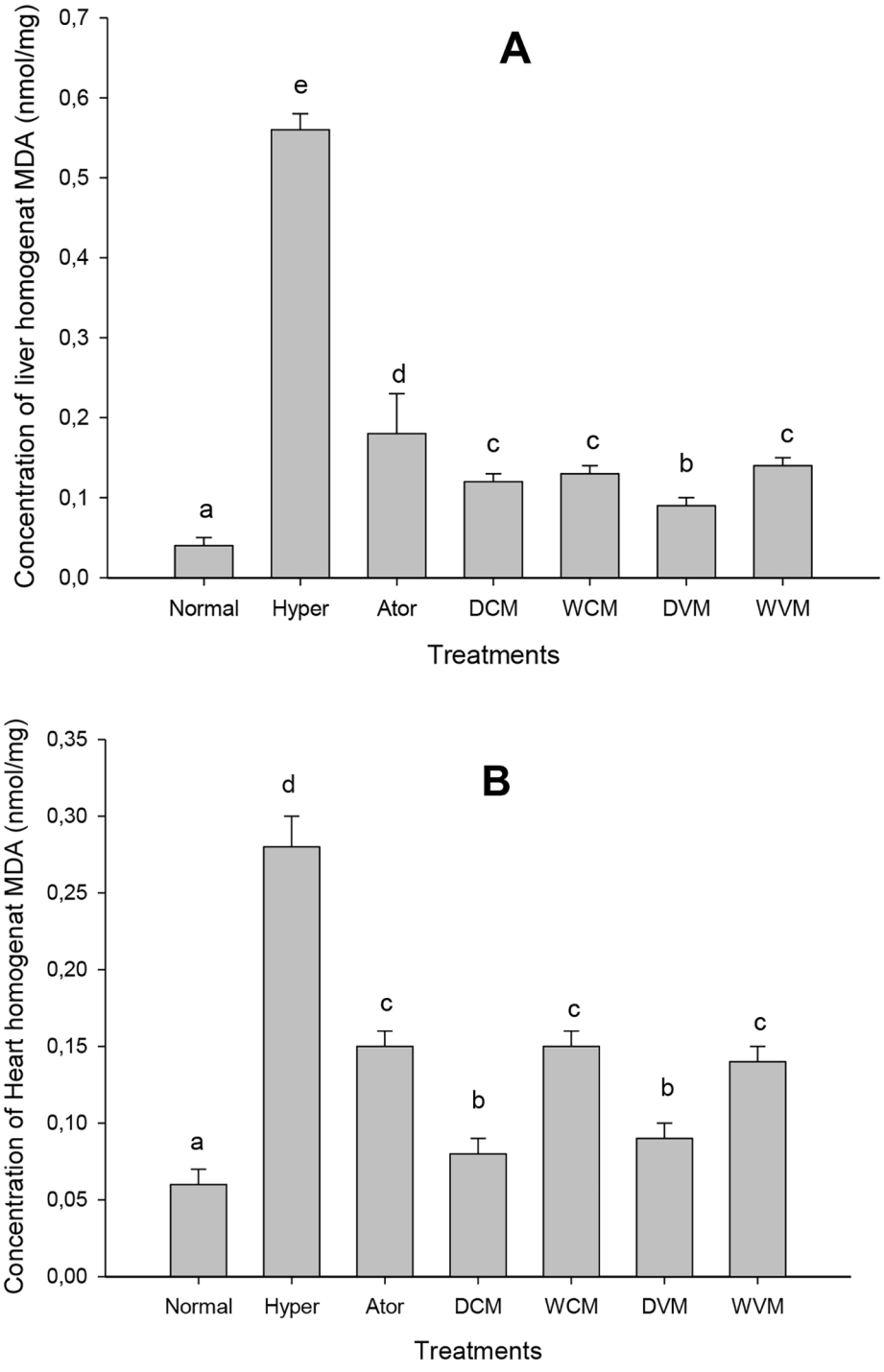

Fig. 2 Effect of *Mucuna pruriens* milk oral administration on level of Malonedialdehyde in liver (**A**) and heart (**B**) homogenats of rats fed with high fat diet Means ± SD (n = 6) followed by different letters are significantly different ($p < 0.05$) as determined by Duncan's multiple range test. DCM: dehulled Cochinchinensis milk group; WCM: whole Cochinchinensis milk group; DVM: Dehulled Veracruz milk group; WVM: whole Veracruz milk group; Normal: Normal control group; Hyper: Hyperlipidemic control group; Ator: Atorvastatin standard control group

(0.89 mg/dL). These findings are consistent with previous studies that have reported that creatinine levels in hyperlipidemic subjects did not change, since muscle mass was similar [38]. This effect may be related to the extract's lipid-lowering potential, as well as to a decrease in liver inflammation, improving dyslipidemia and an increase in responsiveness to leptin and insulin [39]; [40]. In general, hepatocellular injury is assessed by measuring total

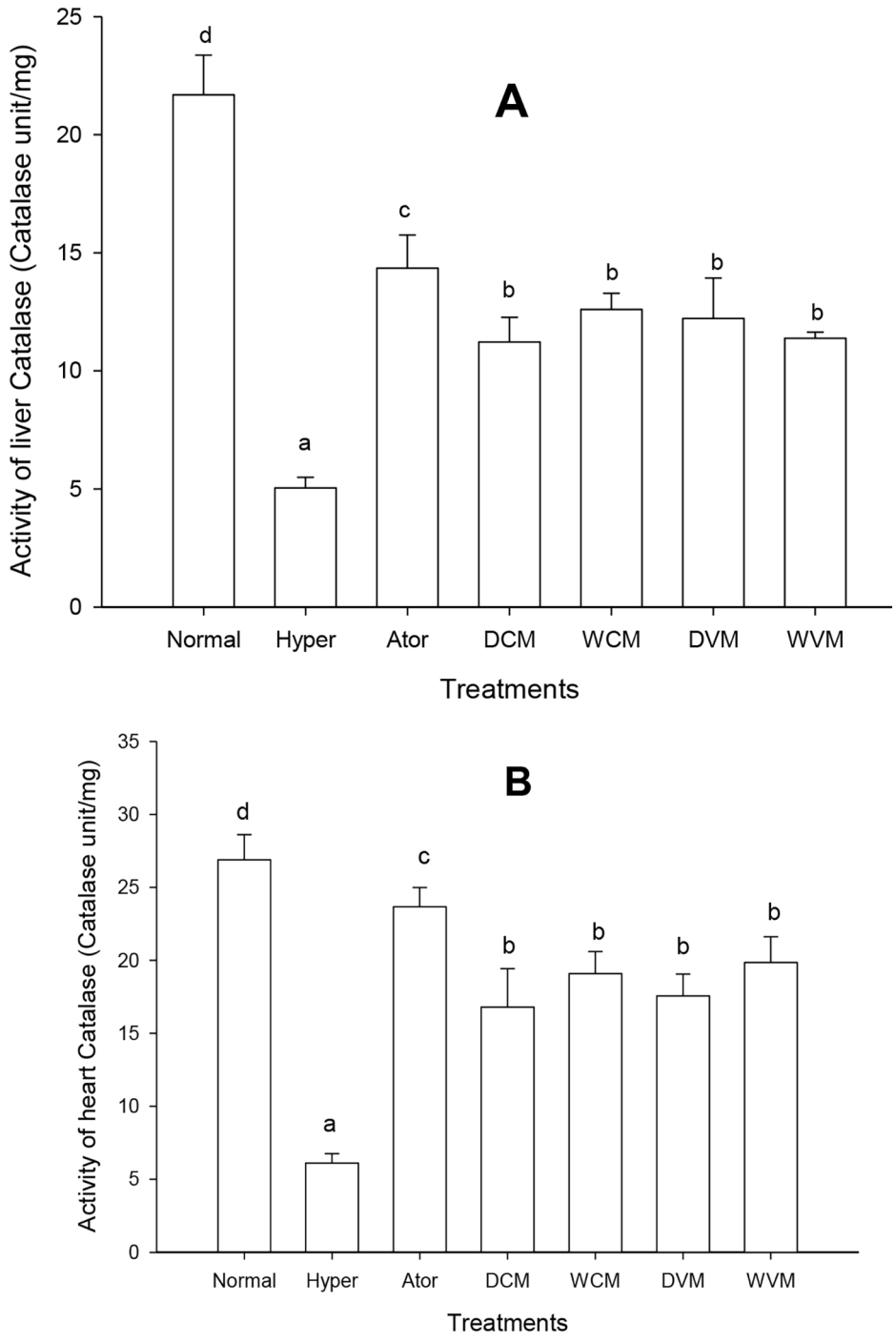

Fig. 3 Effect of *Mucuna pruriens* milk oral administration on catalase activity in liver (**A**) and heart (**B**) homogenats of rats fed with high fat diet Means ± SD (n = 6) followed by different letters are significantly different ($p < 0.05$) as determined by Duncan's multiple range test. DCM: dehulled Cochinchinensis milk group; WCM: whole Cochinchinensis milk group; DVM: Dehulled Veracruz milk group; WVM: whole Veracruz milk group; Normal: Normal control group; Hyper: Hyperlipidemic control group; Ator: Atorvastatin standard control group

protein. It is suggested that a low total protein level may be due to a hepatic or renal disorder [39]. On the other hand, chronic inflammation or liver infection could lead to a high total protein level [39]. In this study, results showed that there were no significant alterations in total protein levels. Therefore, Mucuna milk did not adversely affect liver function.

As a preliminary indicator of toxicity, the weight of some organs was recorded upon sacrifice. Although some studies have noted the toxicity of Mucuna seeds related

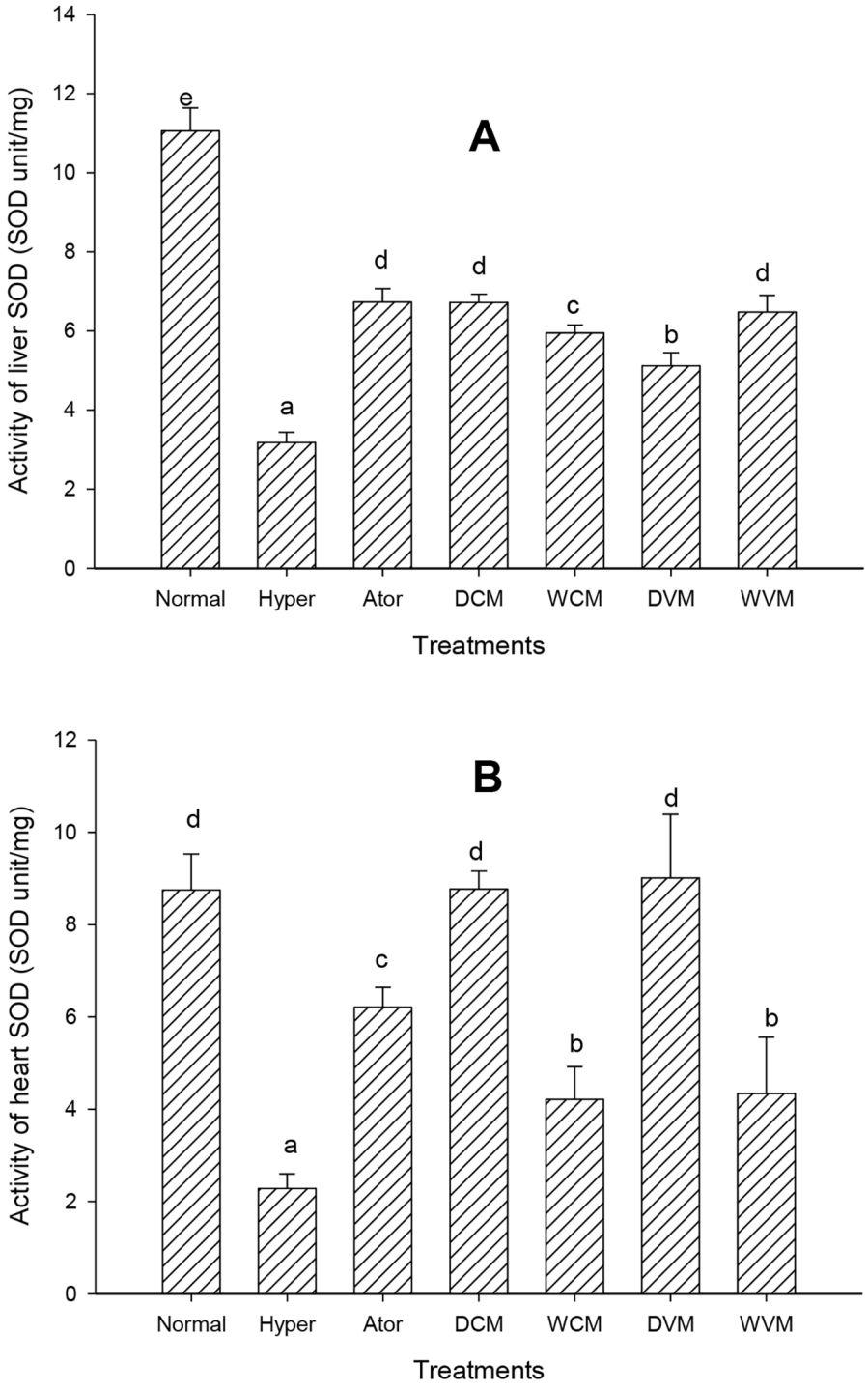

Fig. 4 Effect of *Mucuna pruriens* milk oral administration on SOD activity in liver (A) and heart (B) homogenats of rats fed with high fat diet Means ± SD (n = 6) followed by different letters are significantly different ($p < 0.05$) as determined by Duncan's multiple range test. DCM: dehulled Cochinchinensis milk group; WCM: whole Cochinchinensis milk group; DVM: Dehulled Veracruz milk group; WVM: whole Veracruz milk group; Normal: Normal control group; Hyper: Hyperlipidemic control group; Ator: Atorvastatin standard control group

to their L-Dopamine content [41], no significant findings were recorded, especially in the treated group, suggesting that Mucuna milk was not toxic at the administered dosages, a maximum dosage of 40 mg/kg of milk was used. However, more profound toxicology studies are required in the event that Mucuna milk is further advised to be used for preventing weight gain.

Lipid peroxidation is a free radical mediated process leading to oxidative deterioration of polyunsaturated lipids. Under normal physiological conditions, low concentrations of lipid peroxides are found in plasma and tissues. A significant increases of lipid peroxidation (MDA) in liver and heart of rats fed HFD is probably due to an increase in the generation of free radicals which activate the lipid peroxidation system [32]. The reduction of MDA could be related to the phenolic content of Mucuna milks. As reported previously, Mucuna milks are rich in phenolic compounds [12]; [13]. In fact, phenolic compounds may be responsible for scavenging of free radicals liberated by deterioration of lipids and thus enhance both enzymatic and non-enzymatic antioxidants in hyperlipidemic rats [33].

It is known that high concentrations of lipid peroxidation are associated with decreased antioxidant enzymes such as SOD and catalase, which play an important role in relieving cellular stress [42]. For example, SOD is responsible for the conversion of the superoxide radical into hydrogen peroxide and molecular oxygen, while catalase is used to reduce hydrogen peroxides and protect the upper tissues from highly reactive hydroxyl radicals [43]. The significant decrease in antioxidant enzymes observed in the liver and heart of control hyperlipidemic rats may be attributable to the lack of antioxidant defenses to prevent ROS -mediated damage [44]. The reduction in catalase and SOD activities might be a response to the increased production of H_2O_2 and O_2 by the autoxidation of lipids [45]. In addition, the significant and negative correlations observed between catalase and MDA (r= -0.86; p=0.05); MDA and SOD (r=−0.60; p=0.05) confirms that the induced hyperlipidemia would have caused the peroxidation of antioxidant enzymes in rat. Treatment with Mucuna milks increased the activity of these enzymes and may help to control free radicals when compared to untreated hyperlipidemic rats.

Conclusion

The present study evaluated the anti hyperlipidemic and antioxidant properties of *Mucuna* milks. In conclusion, these vegetables milks offer a promising therapeutic value in prevention of oxidative stress that developed in hyperlipidemia. These effects could be mainly attributed to its antioxidant properties as shown by significant quenching impact on the extent of lipid peroxidation along with, enhancement of antioxidant defense systems in all the tissue selected. Thus, intake of *Mucuna* milk, as drug might have potential benefit in the management and/or treatment of hyperlipidemia. At present, the exact mechanism of action of *Mucuna* milk is not fully known hence, further studies are needed to determine the main active ingredient having anti hyperlipidemic and antioxidant effects.

Abbreviations

ANOVA	Analysis of variance
AOAC	Association of Official Agricultural Chemists
COVID-19	Corona Virus Disease 2019
NADH	Nicotinamide Adenine Dinicleotide + H
NBT	Nitro blue Tetrazolium chloride
PMS	Phenazine Methosulfate
TBA	Thiobarbituric Acid
TCA	Trichloroacetic Acid

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Authors' contributions

MANG YANNICK DIMITRY conceived and designed the study, carried out the experiments, analyzed and discussed the data and wrote the manuscript. BIDJA ABENA MARIE THERESE and DJIOGUE MANEJO JOSIANE EDITH have been involved in revising this manuscript critically for important intellectual content. ABDOU BOUBA Armand, NJINTANG YANOU Nicolas were the major contributors in conceiving and designing the experiment. All authors read and approved the final manuscript.

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Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

The experimental procedures described below were approved by the animal ethical committee of Higher Technical Teachers' Training College of Ebolowa (University of Yaounde I, Cameroon).

Consent for publication

Not applicable.

Conflict of interest

The authors declare that they have no conflicts of interest related to the publication of this study.

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