Original Research Article

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

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ABSTRACT

Aims: to investigate the potential of *Mucuna* milk to influence weight gain, blood lipid levels and redox status in a rat model on a high-fat diet.
Study design:42 healthy male Wistar rats were randomly divided into 7 groups of 6 rats. Group I received a standard diet; Group II was fed a high fat diet only; Group III was fed a high fat diet and treated with Atorvastatin (10 mg/kg per day) orally for 4 weeks; Group IV, V, VI and VII were tests groups fed a high fat diet and given orally 20 mL of vegetable milk.
Methodology:*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 received orally atorvastatin (10 mg/kg/day). In addition, four test groups (IV, V, VI, VII) consisting of rats fed a high fat diet received oral administration of 20 mL of vegetable milk per day (10 mL at morning and 10 mL in the afternoon).
Results: After five weeks, rats on a high-fat diet had an increase in their initial body weight of about 224%, with higher abdominal fat. A significant increase (P<0.05) in lipid peroxidation (MDA) in the liver and heart was also observed.However, oral administration of *Mucuna* milk inhibited weight gain (about 66 % reduction) and abdominal fat (54.53 – 55.60 % reduction). The reduction of LDL, VLDL, Triglycerides and Total cholesterol was remarkable in the groups of rats treated with vegetable milk, as about 67 % of reduction was observed with dehulled *Mucuna* milks (DCM, DVM) and 69 % of reduction with whole *Mucuna* milks (WCM, WVM). The hyperlipidaemic group of rats had higher levels of ASAT (134.17 UI/L) and ALAT (101.72 UI/L). However, *Mucuna* milks improved the ASAT and ALAT levels in rats. The reduction of MDA (70-50 %) was related to phenolic content of *Mucuna* milks. Moreover, 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 showed that treatment with *Mucuna* milks has anti-hyperlipidaemia properties and can increase the activity of antioxidant enzymes.

Keywords: Mucuna milk, high fat diet, serum biochemistry, antioxidant properties

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2 INTRODUCTION

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].

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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 hyperlipidaemic patients are more exposed to COVID-19 complications during the acute phase of the infection over a long period of time.

The development of hypolipidemic drugs or formulation from natural source of natural origin has recently gained importance. Therefore, the study of antioxidant and hypolipidemic activities of foods may provide a new pharmacological approach in the treatment of hyperlipidemia [5]. Many studies have shown that pulses extract and leguminous isolated proteins can reduce elevated serum cholesterol and triglycerides [6],[7] [8]. In addition, vegetable milk produced from common leguminous seeds, such as soya milk and peanut milk, possess hypolipidemic and antioxidant properties that may be useful in reducing the risk of cardiovascular diseases [9]; [10].

In our previous study, we investigated the optimal conditions for the production of *Mucuna* milk [11]; [12]; [13], and also evaluated the chemical composition and protein digestibility of this milk *in vivo* [14]. The obtained results showed that this vegetablemilk is not only a good source of protein, but its consumption can reduce serum cholesterol and triglycerides levels in normal young rats fed diets formulated with this milk as a main protein source [14]. In fact, *Mucuna pruriens* belongs to the Fabaceae family.Its seeds are used as a soup thickener by the rural population in the Far North region of Cameroon and it is also consumed by the Ibos in southeastern Nigeria, the Indian tribal sects, the Mundari and Dravidian groups [15]. The nutritional properties of *Mucuna* seeds are comparable to those of soybeans as they contain similar proportions of protein, lipids, minerals and other nutrients. Therefore, the objective of this study was to investigate whether *Mucuna* milk can affectbody weight gain, blood lipid levels and redox status of rats fed a high-fat diet.

3 MATERIALS AND METHODS

2.1 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.After identification,the samples were assigned a voucher specimen number PQG/I/ 2020/2021/015. *Mucuna* bean flours and vegetable milk samples were produced as previously described by Mang et al. [12], [13],respectively.

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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 and then maintained with controlled temperature water bath. After incubation, the sample was centrifuged at 1500 g and 20 °C for 15 min 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 then kept at 4 °C in a refrigerator for a maximum of 4 hrs. The vegetables milks obtained from *Mucuna Veracruz* flours were coded WVM (Whole *Veracruz* milk) and DVM (Dehulled *Veracruz* milk), whilethat obtainedfrom *MucunaCochinchinensis* flours were coded WCM (Whole *Cochinchinensis*milk) and DCM (Dehulled*Cochinchinensis*milk).

2.2 Animal experiments and biological assay

2.2.1 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 Ebolowa. Forty-two healthy male Wistar rats (three months old, weighing 130 - 145 g) were divided randomly into 7 groups each containing 6 rats. The animals were obtained from the animal house of National School of Agro-Industrial Sciences, Ngaoundéré University, Cameroon and then kept in cages, 1 animal per

cage, with relative humidity (55 %) in a 12 hrs light/dark cycle at 25 ± 2 °C. Before the experiment, the rats had access to water and a standard diet *ad libitum*.

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As illustrated in Table 1, Group I was made of normal control rats fed with a standard diet; Group II consisted of rats receiving high fat diet only; Group III was composed of rats fed with a 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 groupsconsisting of rats fed with a high fat diet and given orally 20 mL of vegetable milk per day (10 mL in the morning and 10 mL in the afternoon). The animals of groups IV, V, VI and VII were treated with dehulled*Cochinchinensis*milk (DCM), whole *Cochinchinensis*milk (WCM), dehulled Veracruz milk (DVM) and whole *Veracruz* milk (WVM), respectively. All rats received water and their experimental diets *ad libitum*.

Table 1. Description of treatment administrated to experimental rats

Groups	Treatments
Group I	Fed with a normal diet (Normal control group)
Group II	Fed with a High fat diet (Hyperlipidaemic control group)
Group III	Fed with a High fat diet + Atorvastatin (10 mg/kg/day) (Atorvastatin standard control group)
Group IV	Fed with a 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 a 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 a High fat diet + received oral administration of 20 mL of dehulled*Veracruz* milk per day (10 mL at morning and 10 mL at afternoon) (DVM group)
Group VII	Fed with a High fat diet + received oral administration of 20 mL of whole *Veracruz*milk per day (10 mL at morning and 10 mL at afternoon) (WVM group)

The compositions of the standard and high fat diet were as follow:
Standard Diet (SD): Cassava starch 60 %, Sucrose 5 %, Casein 10 %,sunflower 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 %, sunflower oil 10 %, salt mixture with starch 5 %, coconut oil 25 %, cellulose 5 %, Vitamin mixture 4 % and mineral mixture 1 % [16].

2.2.2 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 follow:

(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. To evaluate the mean of fecal lipids using soxhlet method, fecal samples were recorded at the beginning of the experiment (week 1), at the middle (week 2) and then at the final stage (week 4) of the study [17].

2.2.3 Blood sampling and biochemical analysis

After 12 hours of the last administration, overnight-fasted animals were anaesthetized by inhalation of isoflurane impregnated on a cotton wool and then 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. Then, clear serum was aspirated, stored frozen and used for further analysis.
Total cholesterol (TC), triglycerides and high-density lipoprotein cholesterol (HDL-c) contents were determined as previously described [18],[19],[20].The analysis of glucose, creatinine, aspartate aminotransferase (AST)and alanine aminotransferase (ALT) was performed by using commercial kits(Randox Laboratories, UK), following the producer instructions [21].
The concentration of serum low density lipoprotein cholesterol (LDL-c) and very low-density lipoprotein cholesterol (VLDL-c) were determined using Friedwald formula:
LDL-c = TC–(HDL-c + VLDL-c) and VLDL-c = TG/5[22].

2.2.4 *In vivo* analysis of the antioxidant activity

To evaluate the antioxidant activity*in vivo*, 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 for analysis.
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 then 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 previously described method [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 previously described[25]. 0.5 mL of tissue homogenate was mixed with 1 mL of distilled water, and 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 contained 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.The 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.

2.3 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 statisticallyanalyzed 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.

3. RESULTS

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

As illustrated in Figure 1, rats on a 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 HFDgroup showed no significant difference ($p < 0.05$) in weight gain compared to rats receiving atorvastatin. Moreover, compared to rats receiving a HFD, we noted that vegetable milks inhibited weight gain by about 66 %, and whole *Veracruz* milk (WVM) seems to be the most effective because it inhibited weight gain by about 70 %.

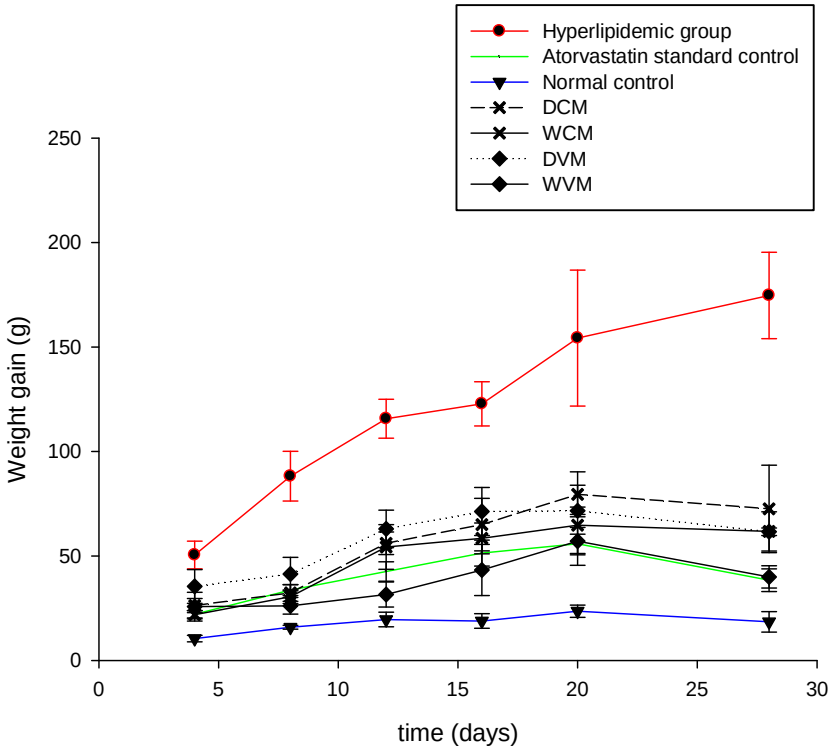

Fig. 1. Effect of *Mucuna* milk oral administration on weight gain of rats fed with high-fat
Results are the means ±SD (n=6) of six animals.DCM: dehulledCochinchinensis milk; WCM: whole Cochinchinensis milk; DVM: Dehulled Veracruz milk; WVM: whole Veracruz milk.

In terms of food intake (Table 2), rats fed with a normal diet consumed more foods (22.60 g) but have lower weight gain compared to the HFD group which only consume about 16.42 g and have higher abdominal weight gain. Oral administration of *Mucuna* milk enhanced significantly food intake in the HFD group ($P< 0.05$). In the other words,*Mucuna* milk induced a significant increase in food intake ($P< 0.05$), of about 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, as abdominal fat of rats on the HFD and whole *Mucuna* milk (WCM and WVM) was significantly reduced ($P< 0.05$) (54.53 – 55.60 %) compared to rats on a HFD only.

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]
Hyperlipidaemic 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: dehulledCochinchinensis milk group; WCM: whole Cochinchinensis milk group; DVM: Dehulled Veracruz milk group; WVM: whole Veracruz milk group.

3.2 Effect of *Mucuna pruriens* milk on serum biochemistry

As shown in Table 3, oral administration of *Mucuna* milk positively influenced (*P*< 0.05) the lipid profiles in the treated group receiving a high fat diet. Moreover, dehulled*Mucuna* seeds appeared to significantly (*P*< 0.05) affect the effectiveness of these vegetable milks. Total cholesterol reduction was remarkable in groups of rats treated with vegetable milk, as about 67 % reduction was obtained for dehulled *Mucuna* milks (DCM, DVM) and 69 % reduction for whole *Mucuna* milks (WCM, WVM). A reduction of LDL, VLDLand Triglycerides level was also recorded in the treated groups.

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	Hyperlipidaemic 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: dehulledCochinchinensis milk group; WCM: whole Cochinchinensis milk group; DVM: Dehulled Veracruz milk group; WVM: whole Veracruz milk group.

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It's observable in Table 4 thatHFD group had significant (P< 0.05) higher blood glucose levels (278.11±14.49mg/dL) at the end of the experimentation compared to rats receiving a normal diet (81.51±1.35 mg/dL).The administration of *Mucuna* milk improved blood glucose, dependently of seeds type, 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 bothrats fed a HFD (0.88 mg/dL) or a normal diet (0.89 mg/dL).

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 the experiment	End of experiment
Normal control	82.5±1.16[a]	81.51±1.35[a]
Hyperlipidaemic 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: dehulledCochinchinensis milk group; WCM: whole Cochinchinensis milk group; DVM: Dehulled Veracruz milk group; WVM: whole Veracruz milk group.

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In this study, three markers of liver function were measured (ASAT, ALATand Total Protein). As shown in Table 5, significant changes (P< 0.05) were observed in the ASAT and ALAT levels of rats. Hyperlipidaemic group rats had significantly (p < 0.05) higher ASAT (134.17 UI/L) and ALAT (101.72 UI/L) levels than normal ratswith 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. Therefore, this vegetable milk reduced hepatic liver injury, as reflected by decreased levels of ALT and AST.

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]
Hyperlipidaemic 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: dehulledCochinchinensis milk group; WCM: whole Cochinchinensis milk group; DVM: Dehulled Veracruz milk group; WVM: whole Veracruz milk group.

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Results in Table 6 shown that no significant findings were recorded, especially in the treated group, suggesting that *Mucuna* milk was not toxic at the administered dosages.

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]
Hyperlipidaemic 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: dehulledCochinchinensis milk group; WCM: whole Cochinchinensis milk group; DVM: Dehulled Veracruz milk group; WVM: whole Veracruz milk group.

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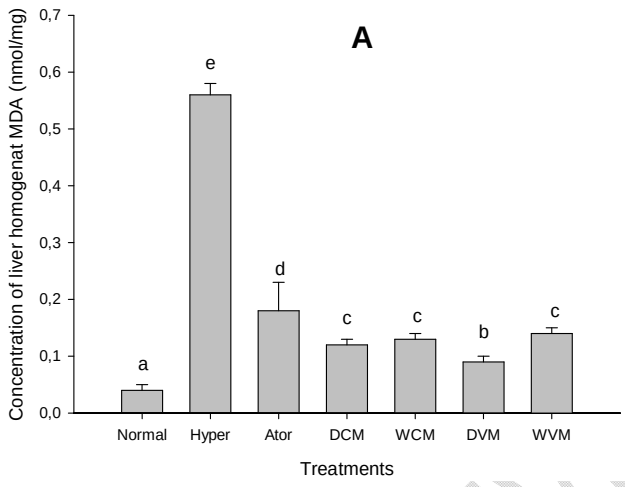
3.3 Antioxidant potential of *Mucuna* milk in rats fed hyperlipidaemic diet

Figure 2 shows that consumption of a high fat diet induced a significant ($p < 0.05$) increaseof lipid peroxidation (MDA) in liver and heart. Significant decreased levels ($p < 0.05$) of lipid peroxidation in the administration of *Mucuna* milks in tissues of rats fed a high fat diet was observed when compared to normal control rats (Figure 2). MDA reduction was about 70 % for liver and 50 % for heart.



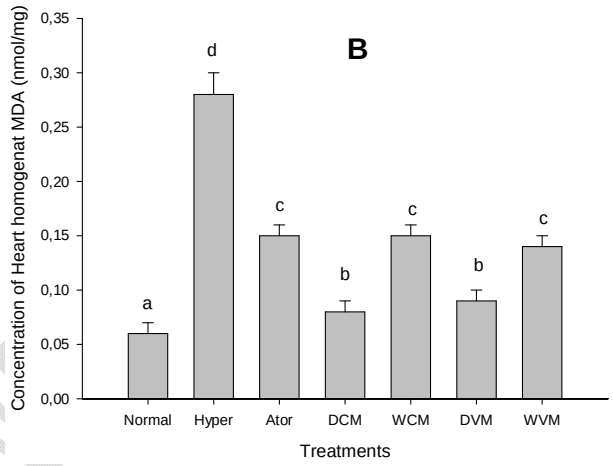


Fig. 2. Effect of *Mucuna pruriens* milk oral administration on level of Malonedialdehyde in liver (A) and heart (B) homogenates of rats fed with high fat diet.

Means ±SD (n=6) followed by different letters are significantly different (P< 0.05) as determined by Duncan's multiple range test. DCM: dehulledCochinchinensis milk group; WCM: whole Cochinchinensis milk group; DVM: Dehulled Veracruz milk group; WVM: whole Veracruz milk group; Normal: Normal control group; Hyper: Hyperlipidaemic control group; Ator: Atorvastatin standard control group.

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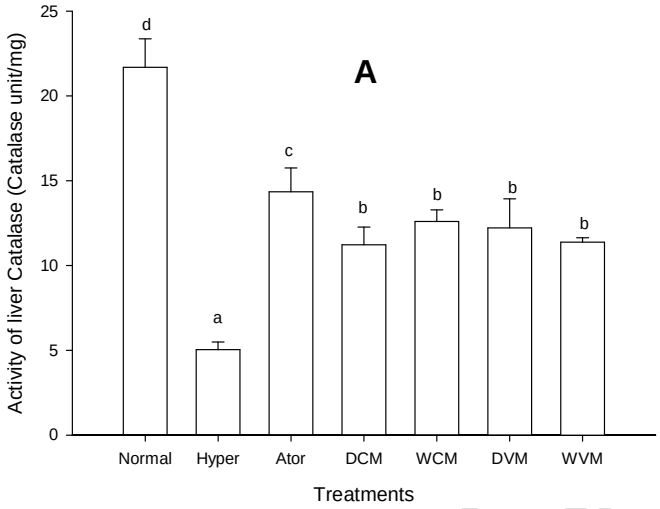
As illustrated in Figures 3 and 4, activities of SOD and Catalase, antioxidants decreased significantly ($p < 0.05$) in liver and heart of hyperlipidemia control rats. There was no significant difference on the effect of different milks on Catalase activity (Figure 3). However, as observed in Figure 4, vegetable milks produced from undehulled*Mucuna* seeds increased more the activity of SOD in the heart.



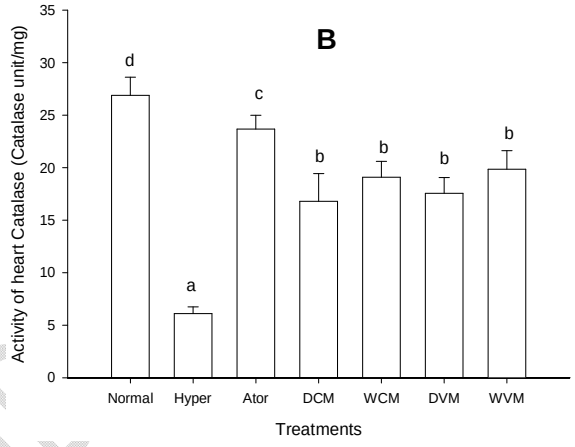


Fig. 3. Effect of *Mucuna pruriens* milk oral administration on catalase activity in liver (A) and heart (B) homogenates of rats fed with high fat diet.

Means ±SD (n=6) followed by different letters are significantly different (P< 0.05) as determined by Duncan's multiple range test. DCM: dehulledCochinchinensis milk group; WCM: whole Cochinchinensis milk group; DVM: Dehulled Veracruz milk group; WVM: whole Veracruz milk group; Normal: Normal control group; Hyper: Hyperlipidaemic control group; Ator: Atorvastatin standard control group.

Comment [D13]: Whatismeaninga,b.c

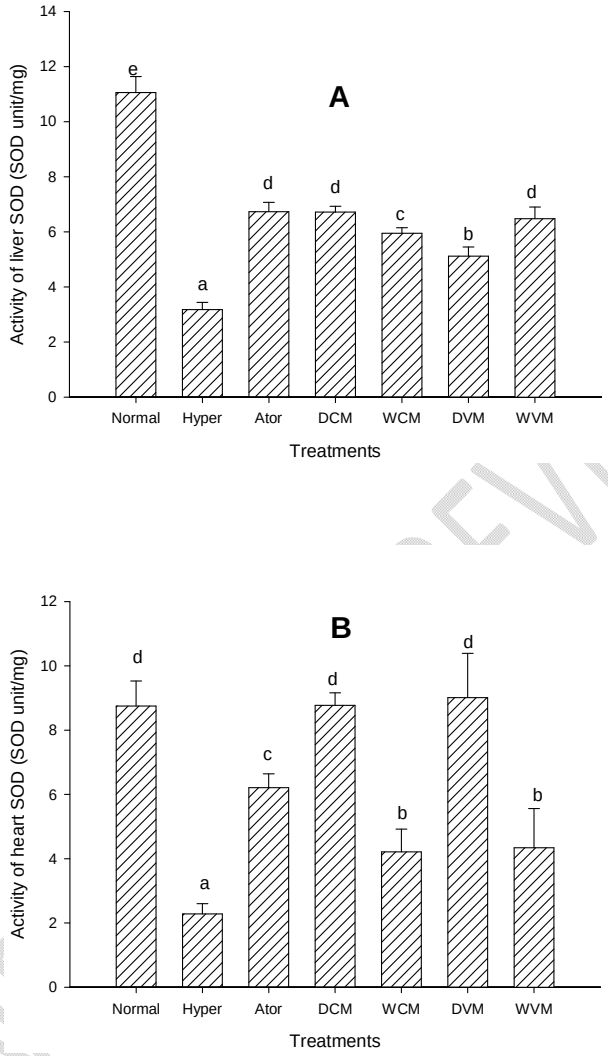


Fig. 4. Effect of *Mucuna pruriens* milk oral administration on SOD activity in liver (A) and heart (B) homogenates of rats fed with high fat diet.

Means ±SD (n=6) followed by different letters are significantly different (P< 0.05) as determined by Duncan's multiple range test. DCM: dehulledCochinchinensis milk group; WCM: whole Cochinchinensis milk group; DVM: Dehulled Veracruz milk group; WVM: whole Veracruz milk group; Normal: Normal control group; Hyper: Hyperlipidaemic control group; Ator: Atorvastatin standard control group.

Comment [D14]: Whatismeaninga,b.c

4. 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-hyperlipidaemic 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-hyperlipidaemic 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, hyperlipidaemia 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 hyperlipidaemic rats, but the changes 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 a HFD and those fed a normal diet. These findings are consistent with previous studies that have reported that creatinine levels in hyperlipidaemic 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 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 is related 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 (40 mg/kg of milk). However, more profound toxicology studies are required to confirm that Mucuna milk can 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 increase of lipid peroxidation (MDA) in liver and heart of rats fed a 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 free radicals liberated during the deterioration of lipids and thus enhance both enzymatic and non-enzymatic antioxidants in hyperlipidaemic rats [42].

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 [43]. 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 [44]. The significant decrease in antioxidant enzymes observed in the liver and heart of control hyperlipidaemic rats may be attributable to the lack of antioxidant defenses to prevent ROS -mediated damage [45]. 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 [46]. 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 hyperlipidaemic rats.

5. CONCLUSION

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

ETHICAL APPROVAL

All authors hereby declare that all experiments described above have been examined and approved by the animal ethical committee of Higher Technical Teachers' Training College of Ebolowa (University of Ebolowa, Cameroon).

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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.