Hypolipidemic and antioxidant effects of vegetal milk produced with *Mucuna pruriens* L. seed in rats fed a high-fat diet

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**ABSTRACT**

Research on the production of lipid-lowering drugs from natural sources has become increasingly important. In this study, we investigated the hypolipidemic properties of the vegetable milk produced from the seeds of *Mucuna pruriens* L. Vegetable milk were produced from whole or dehulled seeds of two varieties of *M. pruriens*, var. Veracruz: dehulled Veracruz milk (DVM) and whole Veracruz milk (WVM); and var. Cochinchinensis: dehulled Cochinchinensis milk (DCM) and whole Cochinchinensis milk (WCM). Then, the phenolic and antioxidant properties of these milks were analyzed. Furthermore, the effect of the intake on selected antioxidant and serum toxicity markers was assessed on hyperlipidemic rats. Three controls were made of normal rats, untreated hyperlipidemic rats, and hyperlipidemic rats treated with atorvastatin (10 mg/kg/day). Four test groups were made of hyperlipidemic rats and treated for 4 weeks with a diet in which vegetable milk (DCM, WCM, DVM, and WVM) was used as the protein source instead of casein. The antioxidant activity (DPPH free radical scavenging 167.2–299.9 mg/100 ml) and phenolic compounds (122.8–199.5 mg/100 ml) were higher in milks produced with whole seeds than in milks produced with dehulled seeds. Moreover, the levels of creatinine, ALAT, ASAT, and MDA, as well as total, LDL, and VLDL cholesterol, significantly increased in the serum of hyperlipidemic rats (*p* < 0.05). However, the antioxidant enzyme activities of treated rats significantly (*p* < 0.05) decreased compared to those of normal rats. The intake of *M. pruriens* milk significantly reduced the levels of total cholesterol, triglycerides, glucose and LDL in the serum (*p* < 0.05). Whole *M. pruriens* milk exhibited higher antioxidant properties, but showed high levels of ALAT, ASAT, and creatinine, which could have an adverse effect on certain organs. Overall, dehulled *M. pruriens* milk could be a potent hypolipidemic and antioxidant functional food.

1. Introduction

The major causes of cardiovascular disease and heart-related deaths are hyperlipidemia and atherosclerosis, due to high levels of serum cholesterol [1, 2]. Therefore, reducing serum cholesterol levels could also substantially reduce the risk of coronary heart disease [3]. The main lipid-lowering medications currently used are statins. However, there is an increasing number of patients on this drug who suffer from serious side effects, especially hyperuricemia, or do not respond properly to treatment [4]. Therefore, new approaches to controlling the global hyperlipidemia epidemic are still needed.

Over the past few years, the need to develop a naturally occurring hypolipidemic drug or a formulation has become even more important. A lot of studies have been conducted on the effect of foods’ antioxidant and hypolipidemic properties on hyperlipidemia treatment. For instance, it has been shown that protein extracts from pulses and legumes can reduce high levels of serum cholesterol and triglycerides [5, 6, 7, 8]. In addition, several studies have shown that the risk of cardiovascular disease can be reduced by the hypolipidemic and antioxidant properties of certain
milk, such as soy milk and peanut milk, obtained from common legume seeds [9, 10].

In our previous study, we investigated the optimal conditions for the production of Mucuna pruriens milk and its chemical composition as well as its protein digestibility [11, 12, 13, 14]. The results showed that M. pruriens milk is a good source of protein, and its consumption decreased serum cholesterol and triglycerides in normal young rats fed with this milk as the main protein source [14]. Thus, the assessment of the hypolipidemic effect and antioxidant properties of M. pruriens milk is important and should be further investigated, given that M. pruriens milk could be used as a functional food.

M. pruriens is a plant that belongs to the family of Fabaceae. Its seeds are used as a soup thickener by people in the Far North region of Cameroon. Their seeds are also consumed by the Ibos people from the southeastern part of Nigeria, the Indian tribal groups, the Mundari, and the Dravidian group [15]. M. pruriens seeds are similar to soybeans in terms of nutritional properties as they have similar protein, fat, mineral, and other nutrient contents. These seeds have traditionally been used as a tonic and aphrodisiac to enhance male virility. Moreover, it is believed that these seeds also have anti-diabetic, analgesic, and anti-inflammatory properties [16, 17]. Many M. pruriens seeds extract preparations are used to treat several diseases caused by free radicals, including aging, rheumatoid arthritis, diabetes, male infertility, and neurological diseases.

Although the seeds of M. pruriens have already been the subject of several studies, there are no reports on the hypolipidemic activity of M. pruriens milk. Thus, the objectives of this study are (1) to investigate the hypolipidemic and antioxidant activity of M. pruriens milk in rats fed a high-fat diet, (2) to determine the effect of post-processing on dehulled and whole M. pruriens milk, and (3) to determine the differences in bioactivities of the two varieties of M. pruriens milk studied (Veracruz and Cochinchinensis).

2. Materials and methods

2.1. Sampling and production of M. pruriens milk

The two varieties of Mucuna pruriens L. seeds (var. Cochinchinensis and var. Veracruz mottle) used for this study were obtained from the International Institute of Tropical Agriculture (IITA) of Yaoundé, Cameroon. M. pruriens bean flour and vegetable milk samples were produced as previously described by Mang et al. [12, 13]. The slurry was prepared by mixing 8 g of M. pruriens flour with 100 mL of distilled water, and then centrifuged at 3500 rpm for 60 min, using an electric stirrer (a TECHNICON stirrer motor, England) under an extraction temperature of 60 °C, which was maintained for a controlled temperature water bath. After the incubation time, the sample was centrifuged at 1500 g for 15 min at 20 °C by using a 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 further analysis within a maximum of 4 h. The vegetable milks resulting from Veracruz variety flours was named WVM (Whole Veracruz milk), and DVM (Dehulled Veracruz milk), while that from Cochinchinensis variety flours were coded WCM (Whole Cochinchinensis milk) and DCM (Dehulled Cochinchinensis milk).

2.2. Phenolic compounds of M. pruriens milk

The total phenolic compound of the different samples was determined colorimetrically based on the Folin-Ciocalteau reagent as gallic acid equivalents, as described earlier by Gao et al. [18]. The tannin level (equivalent of milligrams of tannic acid per 100 mL) of milk was determined by using the method described by Bainbridge et al. [18]. Flavonoid content (equivalent of mg quercetin per 100 mL) was assessed as previously described by Mimica-Dukic et al. [19].

2.3. In vitro antioxidant activity of M. pruriens milk

The reducing power of milk was measured based on previous method described by Duh and Yen [20], and the results were expressed as grams of ascorbic acid per 100 mL of milk. The DPPH free radical scavenging activity was determined following the method described by Okade et al. [21], and the results were expressed as a decreased percentage of absorbance based on the control values. Moreover, the DPPH activity was compared with that of Trolox, which was used as a standard sample. Furthermore, ferrous ion chelating capacity (%) was measured according to Decker and Welch [22].

2.4. Animal experiments and biological assays

2.4.1. Experimental animals

In this study, healthy male Wistar rats (7–8 weeks old, 135–150 g weight) were provided by the animal house of the National School of Agro-Industrial Sciences of the University of Ngaoundéré, Cameroon. These animals were kept in individual cages where the humidity was maintained at 55% with a 12-hour light/dark cycle at a temperature of 25 ± 2 °C. The animals had access to water and a standard diet ad libitum during the entire two-week acclimatization period. All experiments were performed following the guidelines established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and ethically approved by the Institutional Committee of the Ministry of Scientific Research and Innovation of Cameroon (register number: PV 2107).

2.4.2. Induction of hyperlipidemia

The high-fat diet was prepared and administered for 5 weeks, except for the normal control group, which was fed the standard diet. The fecal material was recorded daily and used to determine the amount of lipids excreted using the soxhlet method [23]. At the end of the fifth week, the total cholesterol level in the serum was estimated and all rats with a level greater than 250 mg/dl were selected and considered as hyperlipidemic rats based on the method described by Shinnick et al. [24]. The compositions of the two diets were as follows:

**Standard Diet:** 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 Diet:** 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%.

2.4.3. Experimental design

The animals placed in individual metabolic cages were randomly grouped into seven groups, each containing six rats. The average weight of all animals was 140.12 ± 3.02 g, and there was no significant (p < 0.05) difference in the average weight of each group of rats. As shown in Table 1, Group I consisted of normal control rats fed a standard diet; Group II of hyperlipidemic rats fed a standard diet; Group III of hyperlipidemic rats fed a standard diet and treated with standard drug, Atorvastatin (10 mg/kg), orally for 4 weeks; Group IV, V, VI, and VII were test groups consisting of hyperlipidemic rats fed a standard diet for 4 weeks with vegetable milk as the protein source. The volume of milk necessary to achieve 10% protein in the diets was calculated based on the proximate composition of these milks previously reported in our studies [13, 14]. In this vein, the quantity of milk corresponding to 100 g of diet was 85 mL, 160 mL, 85 mL and 160 mL in groups IV, V, VI, and VII, for dehulled Cochinchinensis, whole Cochinchinensis, dehulled Veracruz and whole Veracruz milks, respectively. Around 20 g of diet were served to every rat daily, after 24 h the residues were weighed in order to evaluate the food intake (Table 1).

2.4.4. Blood sampling and biochemical analysis

Overnight-fed animals were anesthetized and sacrificed 12 h after the last administration by inhaling isoflurane impregnated on absorbent
Means ± SD (n = 6) followed by different letters in the same column are significantly different (p < 0.05) as determined by Duncan’s multiple range test.

cotton [25]. Abdominal fat was also carefully dissected and weighed. The blood was collected by cardiac puncture into a vacuum tube and centrifuged at 3000 rpm for 10 min. The clear serum was then collected and stored under freezing conditions before being used for further analysis. Furthermore, the serum was analyzed for total cholesterol (TC) [26], triglycerides [27], high-density lipoprotein cholesterol (HDL-c) [28], glucose, creatinine, aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) [29] by using commercial kits (Randox Laboratories, UK) according to standard procedures described by the manufacturer. The concentrations of low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) in the serum were determined using the Friedwald Eqs. (1) and (2) as follows:

\[
LDL-c = TC - (HDL-c + VLDL-c) \quad (1) 
\]

and

\[
VLDL-c = TG/5 \quad (2)
\]

Likewise, the atherogenic index (A.I.) and protection percentage were determined with Eqs. (3) and (4) as previously reported [31].

\[
A.I. = \frac{LDL-c + VLDL-c}{HDL-c} \quad (3)
\]

Protection (%) = $\frac{A.I. \text{ of negative control group} - A.I. \text{ of treated group}}{A.I. \text{ of negative control group}} \times 100 \quad (4)$

2.4.5. In vivo analysis of the antioxidant activity

Lipid peroxidation in serum was estimated by colorimetric quantification of malondialdehyde (MDA) [32]. In this method, 0.1 mL of serum was treated with 2 mL of TBA-TCA-HCl reagent (TBA 0.37%, 0.25 N HCl, and 15% TCA) in a 1:1:1 ratio, then placed in a water bath for 15 min before cooling. The absorbance of clear supernatant was measured at 535 nm against a blank. The lipid peroxidation was calculated on the basis of the molar extinction coefficient of MDA and expressed as nmol of MDA/g of protein.

The catalase activity in the serum was determined by a previously described method [33]. The reaction mixture contained 1.0 mL of 0.01 M phosphate buffer pH 7.0, 0.1 mL of serum, and 0.4 mL of 2 M of H2O2 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 was expressed as mmol of H2O2 consumed/min/mg protein.

Superoxide dismutase (SOD) activity in serum was assayed by the method of Kakkar and Viswanathan [34]. Then, 0.5 mL of serum 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 was centrifuged to collect the supernatant. The mixture contains 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 mM NBT, 0.2 mL of 780 μM NADH, appropriately diluted enzyme preparation, and water in a total volume of 3 mL. NADH was added to initiate the reaction. The mixture was then incubated at 30 °C for 90 s. After the incubation time, 1 mL of glacial acetic acid was added to stop the reaction. Afterwards, 4 mL of n-butanol were added and the reaction mixture was stirred and shaken vigorously. The chromogen intensity in the butanol layer was measured at 560 nm against a blank that contains butanol. As a control, the test mixture without enzyme was used. A unit of enzyme activity is defined as the enzymatic reaction that results in 50% inhibition of NBT reduction within 1 min under the test conditions.

2.5. Statistical analysis

The data reported in the tables and figures was carried out in triplicate or more replicated determinations. All data were expressed as mean standard deviation and statistically analyzed using two-way ANOVA. When statistical differences were found, the Duncan’s Multiple Range Test was applied in order to classify samples at a significant level of 5%. The Statgraphics Program (Statistically Graphics Educational, version 6.0, 1992, Manugistics, Inc. and Statistical Graphics Corp., USA) was used for the statistical analysis. Using Minitab 18 Software, a principal component analysis was used to generate a Pearson correlation matrix.

3. Results

3.1. Phenolic compounds and in vitro antioxidant activities of Mucuna milk

The phenolic compound content and some antioxidant properties of M. pruriens milk samples are shown in Table 2. The total phenolic content...
ranged from 122.8 ± 2.8 mg to 178.8 ± 2.1 mg in dehulled *M. pruriens* milk and from 135.3 ± 1.8 to 199.5 ± 2.8 mg in whole *M. pruriens* milks. The content of tannins determined varied significantly (p ≤ 0.05) between the different varieties of *M. pruriens* milks. Overall, tannin content in milk with whole *M. pruriens* seeds (2.09 ± 0.05–2.15 ± 0.07 mg per 100 mL) was 80% higher than that in dehulled *M. pruriens* milk (0.27 ± 0.02–0.43 ± 0.02). The content of total phenolic and flavonoids was much higher in the variety Veracruz than in the variety Cochinchinensis (Table 2). According to the data presented above, removing seed hulls is associated with a decrease in total phenolic compounds, flavonoids, and tannins in this vegetable milk. Dehulling and the varieties of *M. pruriens* have similar effects on the phenol content, the total reducing power and DPPH scavenging power, while only dehulling significantly decreases the chelating power (p < 0.05) (Table 2). On the other hand, the reducing power of the varieties of milk ranged from 725.66 to 964.33 mg of Vitamin C per 100 mL of milk. However, dehulling reduced the reducing power by up to 11–15%. The DPPH free radical ranged from 167.2 to 299.9 mg of Trolox/100 mL.

### 3.3. In vivo antioxidant activity of *M. pruriens* milk

The serum lipid profile of rats that were fed different diets is shown in Table 3. As shown in that table, rats fed a normal diet regime exhibited the lowest total LDL and VLDL cholesterol, and triglyceride levels. However, hyperlipidemic rats fed a normal diet for 4 weeks showed the highest total LDL and VLDL cholesterol and triglyceride contents, but had the lowest HDL cholesterol. In addition, overweight rats had the highest abdominal fat (Figure 1). In this study, compared to normal rats, the total cholesterol, LDL, VLDL, and triglycerides levels in rats’ serum increased in rats fed high fat by 146, 142, 153, and 156%, respectively, while the HDL cholesterol decreased by 40%. The administration of atorvastatin for 4 weeks to overweight rats fed a normal diet regime (group III) led to a significant decrease in serum total cholesterol (33%), LDL-C (49%), VLDL-C (52%) and triglycerides (53%), while no significant changes were observed in HDL-C. Moreover, compared to untreated HFD rats, overweight rats fed *M. pruriens* milk as a protein source for 4 weeks’ diets showed similar reduction in serum total cholesterol (25%), LDL-C (49%), VLDL-C (38%), and triglycerides (38%). Dehulling the seeds improves this lipid lowering activity, which seems to be affected by the seed variety. In this vein, Veracruz milk seems to be more effective in lowering lipid levels. However, the level of HDL-C (89%) increased. It’s worth noting that the level of HDL-C in rats fed Mucuna milk was higher than in HFD rats fed a standard diet, regardless of *M. pruriens* milk variety or treatment. Overall, overweight rats lose up to 29% of abdominal fat when treated with atorvastatin, whereas more than a 40% reduction was observed in rats fed *M. pruriens* milk as a protein source (Figure 1).

### 3.3. In vivo antioxidant activity of *M. pruriens* milk

As shown in Figure 2, a notable increase in MDA levels in serum was observed after the induction of hyperlipidaemia compared to normal rats. Compared to HFD rats, when hyperlipidemic rats were submitted to *M. pruriens* milk as a protein source, a reduction of 67% of serum MDA was observed in dehulled *M. pruriens* milk, while 75% was obtained in whole *M. pruriens* milk. Whole Cochinchinensis milk (WCM) exhibited a higher reduction in MDA compared to the other milk. However, no statistically significant difference was found between DVM, DCm, and WVM milks. The reduction of serum MDA levels in animals does not appear to be affected by seed variety or dehulling. Figure 3 shows the change in

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**Table 3. Consumption of *Mucuna pruriens* milk affects total cholesterol, triglycerides, HDL, LDL, and VLDL levels in hyperlipidemic rats fed a high-fat diet.**

| Serum Parameters  | Normal control | Hyperlipidemic control | Atorvastatin standard control | var. Cochinchinensis | var. Veracruz |
|-------------------|----------------|------------------------|-------------------------------|----------------------|--------------|
|                   |                |                        |                               | DCM                  | WCM          |
| Total Cholesterol | 154.3 ± 5.4a   | 380.7 ± 1.4d           | 251.9 ± 5.0d                  | 312.8 ± 2.36b        | 279.0 ± 1.9b |
|                   |                |                        |                               | 290.0 ± 3.8b         | 254.1 ± 3.8b |
| Triglycerides     | 85.6 ± 3.5a    | 219.2 ± 3.3f           | 103.5 ± 4.5d                  | 146.3 ± 4.5e         | 130.7 ± 3.8b |
|                   |                |                        |                               | 160.7 ± 5.5b         | 100.7 ± 1.8b |
| HDL-C (mg/dL)     | 104.7 ± 2.0b   | 62.3 ± 3.3a            | 63.2 ± 2.8a                   | 116.9 ± 1.9f         | 118.5 ± 3.9d |
|                   |                |                        |                               | 122.6 ± 1.6f         | 114.9 ± 3.2f |
| LDL-C (mg/dL)     | 32.5 ± 4.3a    | 273.8 ± 6.8b           | 189.2 ± 3.6d                  | 166.6 ± 3.6f         | 135.0 ± 2.2b |
|                   |                |                        |                               | 135.2 ± 4.2b         | 119.1 ± 1.0b |
| VLDL-C (mg/dL)    | 17.1 ± 0.7a    | 43.4 ± 0.7f            | 20.7 ± 0.9f                   | 29.4 ± 0.8f          | 26.0 ± 0.7f  |
|                   |                |                        |                               | 32.2 ± 0.9f          | 20.2 ± 0.5f  |

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 diet; WCM: whole Cochinchinensis milk diet; DVM: Dehulled Veracruz milk diet; WVM: whole Veracruz milk diet.
SOD and catalase activities with food regimes. The activity of these enzymes was significantly shut down in hyperlipidemic rats compared to normal rats, probably as a consequence of an increase in free radicals associated with increased lipid peroxidation. MDA levels were found to have significant and negative correlations (r = −0.61, r = −0.68; p < 0.05) with SOD and catalase in the groups Atov, DCM, WCM, DVM, and WVM. SOD activity increased 1.76–1.98 times more in rats fed dehulled seed milks (DVM and DCM) compared to HFD rats, and 1.14–1.42 times more in rats fed whole seed milks (WVM and WCM). The opposite effect seems to be observed for catalase, where WVM and WCM milks would have a more positive effect (2.49–3.36 times) compared to DCM and DVM, which stimulate the activity of this enzyme by 3.01–4.03. When compared to the Cochinchinensis variety, the milk of the Vera Cruz variety has a beneficial impact on catalase activity. The activity of SOD appears to be unaffected by seed variety.

### 3.4. The protective action of *M. pruriens* milk

The protective action referred to the atherogenic index and the percentage of protection of the different diets are shown in Table 4. The atherogenic index increased from 0.47 in the normal diet regime to 5.10 in rats fed a high-fat diet. The atherogenic index of the rats treated with atorvastatin for four weeks decreased to 2.95, which corresponds to a protection of 42. Hyperlipidemic rats fed *M. pruriens* milk exhibited much lower values of the atherogenic index (1.18–1.68) and higher protection (62–77%) compared to rats treated with atorvastatin. Overall, protection was not significantly affected by the variety of *M. pruriens* seeds. But it is much higher when whole seeds are used for milk preparation.

### 3.5. Effect of dehulling on the potential toxicity of *M. pruriens* milk

To evaluate the potential toxic effects of ingesting *M. pruriens* milk, serum markers indicating liver and kidney damage were measured at the end of the experimental period. The enzyme activities shown in Table 5 indicated that aspartate amino transaminase (ASAT) and alanine amino transaminase (ALAT) were elevated in the serum of the rats fed a hyperlipidemic diet compared to normal rats. No positive effects were observed on ALAT and ASAT levels in rat serum fed with atorvastatin, probably because of the drug-related cytotoxicity. However, these markers significantly (p < 0.05) decreased in the serum of rats fed

### Table 4. The effect of *M. pruriens* milk consumption on the atherogenic index and percentage of protection in hyperlipidemic rats fed a high-fat diet.

| Groups                    | Parameters | Atherogenic index | Protection (%) |
|---------------------------|------------|-------------------|----------------|
| Normal control            | 0.47 ± 0.04* | /                 | /              |
| Hyperlipidemic group      | 5.10 ± 0.15  | /                 | /              |
| Atorvastatin standard control | 2.95 ± 0.12* | 42.15 ± 1.04  | /              |
| DCM                       | 1.68 ± 0.08  | 62.15 ± 2.55  | /              |
| WCM                       | 1.34 ± 0.06  | 73.69 ± 1.64  | /              |
| DVM                       | 1.40 ± 0.09  | 72.42 ± 1.71  | /              |
| WVM                       | 1.18 ± 0.03  | 76.81 ± 3.61  | /              |

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

*M. pruriens* milk as a protein source, up to a level equivalent to that of HFD rats fed a normal regime. In addition, the dehulling of the seeds leads to a more significant (p < 0.05) decrease in the activity of these enzymes, while the latter seems to be unaffected by the seed variety.

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**Figure 2.** Effect of *M. pruriens* milk consumption on level of malondialdehyde in serum of hyperlipidemic 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 diet; WCM: whole Cochinchinensis milk diet; DVM: Dehulled Veracruz milk diet; WVM: whole Veracruz milk diet.

**Figure 3.** Consumption of *M. pruriens* milk affects catalase (A) and superoxide dismutase (B) activities in the serum of hyperlipidemic rats fed a 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 diet; WCM: whole Cochinchinensis milk diet; DVM: Dehulled Veracruz milk diet; WVM: whole Veracruz milk diet.

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### Table 5. Effect of dehulling on the potential toxicity of *M. pruriens* milk

| Groups                    | Parameters | Atherogenic index | Protection (%) |
|---------------------------|------------|-------------------|----------------|
| Normal control            | 0.47 ± 0.04* | /                 | /              |
| Hyperlipidemic group      | 5.10 ± 0.15  | /                 | /              |
| Atorvastatin standard control | 2.95 ± 0.12* | 42.15 ± 1.04  | /              |
| DCM                       | 1.68 ± 0.08  | 62.15 ± 2.55  | /              |
| WCM                       | 1.34 ± 0.06  | 73.69 ± 1.64  | /              |
| DVM                       | 1.40 ± 0.09  | 72.42 ± 1.71  | /              |
| WVM                       | 1.18 ± 0.03  | 76.81 ± 3.61  | /              |

Means ± SD (n = 6) followed by different letters in the same column are significantly different (p < 0.05) as determined by Duncan’s multiple range test. DCM: dehulled Cochinchinensis milk diet; WCM: whole Cochinchinensis milk diet; DVM: Dehulled Veracruz milk diet; WVM: whole Veracruz milk diet.
Independently of the seed variety and the dehulling, a significant (p < 0.05) hypoglycemic effect of these vegetable milks was also observed in rats, with about 4.71% of decrease compared to HFD or normal rats. On the other hand, ALAT and ASAT levels remained quite high in the serum of rats fed with whole M. pruriens milk. More importantly, compared to HFD and normal rats, creatinine levels were significantly higher only in the serum of rats fed whole M. pruriens milk as a protein source.

4. Discussion

The M. pruriens milk results are consistent with those reported for other vegetable milks [35, 36]. The total phenolic content of whole M. pruriens milks falls within the range reported for soya milk [37]. The removal of hulls induced up to a 10% reduction in the phenolic content level of milk. This observation is in line with Alonso et al. [38], who reported that phenolic compounds are more concentrated in the beans' coats. Among polyphenols, flavonoids are highly represented in M. pruriens beans [39]. The decrease in milk's reducing power is most likely due to a decrease in phenolic compounds. DPPH free radical scavenging indicates the ability of an extract to scavenge free radicals [40]. Another antioxidant mechanism is to chelate ferrous iron, which is an essential mineral for normal physiology, but excess may result in cellular injury [41]. Since ferrous ions are the most effective pro-oxidants commonly found in food systems, a good chelating effect would be beneficial, and the removal of free iron from circulation could be a promising approach to prevent oxidative stress-induced diseases. In this respect, M. pruriens milk, particularly that made from whole M. pruriens seeds, which shows a good free radical scavenging activity, can be exploited for these properties.

It is known that hyperlipidemia intensifies the production of free radicals, which can consequently accelerate the oxidative degradation of polysaturated fatty acids in membranes and increase the level of lipid peroxides and hydroperoxides [42]. One of the most common biomarkers used for studying oxidative damage of lipids is malondialdehyde (MDA), which is one of the major products of lipid peroxidation [43]. It was shown that, in hyperlipidemic rats, higher serum MDA levels are associated with increased levels of lipid peroxidation and thus stress [44]. However, the increase in serum MDA is not only related to hyperlipidemia. It is also observed in Type 2 Diabetes Mellitus, hypertension, cancers, etc. The degree of peroxidation induced by free radicals depends on the balance between free radical generation and the endogenous antioxidant defense mechanism [45]. It was reported that superoxide dismutase (SOD) and catalase are the two most important enzymes of the enzymatic antioxidant defense system [46]. SOD removes the superoxide anion by converting it into hydrogen peroxide, thus reducing the toxic effect caused by this radical, while catalase breaks down the superoxide anion into hydrogen peroxide [47]. The result of this study suggested an efficient protective potential of M. pruriens milk against the generation of free radicals. M. pruriens milk, mainly those produced with whole seeds, has been shown to contain a high level of polyphenols with high antioxidant properties, which could have protected the antioxidant enzymes of the animals and hence improved their antioxidant status.

Previous research found a significant increase in lipid parameters in plasma and tissue as a result of a high-fat diet [48]. The positive effect of M. pruriens milk on the lipid profile may be due to its content of phenols and, in particular, flavonoids, as reported in previous studies. It has been demonstrated that flavonoids stimulate the activity of serum lipoprotein lipase, resulting in a decrease in plasma triglycerides. HDL is a beneficial lipoprotein synthesized in the intestine and liver that carries cholesterol from peripheral tissue to the liver for excretion, thus performing a protective role against fat accumulation-related diseases [49]. Therefore, M. pruriens milk may act as a hypolipidemic agent by increasing HDL-C and reducing plasma levels of VLDL and LDL cholesterol. The phenols in M. pruriens milk, in particular the tannins, may also interfere with the reabsorption of cholesterol in the intestine, increasing the fecal excretion of steroids. This mechanism of fat reduction was evidenced by the significant increase in fecal lipid observed in rats fed atorvastatin and M. pruriens milk, even though M. pruriens milk had little effect. Lecithin acyl transferase (LCAT), which is activated by flavonoids, has also been reported to be a key enzyme for the regulation of blood lipids. LCAT plays a key role in the incorporation of free cholesterol into HDL (this may increase HDL and transferring it back to VLDL and LDL, which are taken back later in liver cells [50]. Besides, M. pruriens phenols may exhibit biological activity in M. pruriens milk, while M. pruriens protein isolates were recently reported to possess hypolipidemic activity [6]. Previous studies reported a protein content of 14 g/100 mL and 6.0 g/100 mL in M. pruriens milks made from dehulled and whole M. pruriens seeds, respectively [13]. In all cases, the mechanism by which the M. pruriens phenols and proteins act as lowering agents still needs to be investigated.

The atherogenic index is used as an indicator to assess susceptibility to atherogenesis. The atherogenic index is used as an indicator to assess susceptibility to atherogenesis [51]. The low risk of susceptibility of the heart and kidney to oxidative damage observed in this study with the M. pruriens milk diet regime may be due to its total phenols and flavonoids content, which were significantly higher in whole Veracruz M. pruriens milk.

ALAT and ASAT are two excellent markers of liver damage, and their increase in the serum of rats fed hyperlipidemic is probably a result of damage to the integrity of the heart and liver. According to Ioannou et al. [52], elevated levels of serum ALT and AST in the absence of viral hepatitis and alcoholism have been reported to lead to a higher risk of cardiovascular disease. The observation suggested some toxicity related to consumption of milk made from whole M. pruriens seeds. Creatinine is a waste product originating from creatine metabolism in muscle, and its retention in the blood is the result of kidney failure [53]. According to Ngatchic et al. [6], rats fed M. pruriens protein isolates made with dehulled mucuna beans showed no significant changes in ALAT and ASAT, whereas rats fed isolate proteins made with undehulled mucuna beans showed significant increases in both these enzymes and creatinine levels. This probably suggested the role of the hull in the increase in

### Table 5. *Mucuna pruriens* milk consumption affects serum creatinine, ALAT, ASAT, and blood glucose levels in hyperlipidemic rats fed a high-fat diet.

| Groups                        | Parameters | Creatinine (mg/dL) | ALAT (U/L) | ASAT (U/L) | Glucose (mg/dL) |
|------------------------------|------------|--------------------|------------|------------|-----------------|
| Normal control               |            | 0.89 ± 0.05<sup>a</sup> | 22.84 ± 1.44<sup>b</sup> | 19.68 ± 2.88<sup>b</sup> | 81.01 ± 1.35<sup>b</sup> |
| Hyperlipidemic group         |            | 0.86 ± 0.08<sup>a</sup> | 54.64 ± 8.09<sup>a</sup> | 71.40 ± 1.62<sup>b</sup> | 87.50 ± 2.65<sup>a</sup> |
| Atorvastatin standard control|            | 0.89 ± 0.15<sup>a</sup> | 62.82 ± 2.12<sup>a</sup> | 84.37 ± 1.16<sup>a</sup> | 82.50 ± 1.23<sup>a</sup> |
| DCM                          |            | 0.83 ± 0.11<sup>a</sup> | 24.24 ± 1.79<sup>a</sup> | 19.07 ± 1.26<sup>a</sup> | 77.22 ± 1.47<sup>a</sup> |
| WCM                          |            | 1.77 ± 0.07<sup>a</sup> | 29.76 ± 0.45<sup>a</sup> | 34.62 ± 2.13<sup>a</sup> | 77.15 ± 1.66<sup>a</sup> |
| DVM                          |            | 0.89 ± 0.05<sup>a</sup> | 25.51 ± 3.70<sup>a</sup> | 18.27 ± 2.13<sup>a</sup> | 77.25 ± 1.37<sup>a</sup> |
| WVM                          |            | 1.92 ± 0.04<sup>a</sup> | 30.12 ± 1.32<sup>a</sup> | 31.72 ± 0.65<sup>a</sup> | 77.31 ± 1.94<sup>a</sup> |

Means ± SD (n = 6) followed by different letters in the same column are significantly different (p < 0.05) as determined by Duncan’s multiple range test. DCM: dehulled Cochinchenis milk diet; WCM: whole Cochinchenis milk diet; DVM: Dehulled Veracruz milk diet; WVM: whole Veracruz milk diet.
The present study evaluated the hypolipidemic and antioxidant properties of M. pruriens milk. It can be concluded that rats with hyperlipidemia fed with M. pruriens milk as hypothesized in vivo. At present, the exact mechanism of action of M. pruriens milk is not fully known. Therefore, further investigations into this area are still needed to isolate and elucidate the exact structure of the lipid-lowering component in M. pruriens milk. This could help to establish evidence of the involvement of Mucuna milk in the treatment of hyperlipidemia.

Declarations

Author contribution statement

Mang Yannick Dimitry: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Bidja Abéna M. Therese; Djiojoe Manejo J. Edith; Panoyo’A Idkowa Emmanuel: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Abdou Bouba Armand; Njintang Yanou Nicolas: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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