The Potential Effect of Aqueous Extract of *Detarium microcarpum* Bark on Certain Metabolic Disorders Associated with an Atherogenic Diet in Rats

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Abstract

Atherosclerosis is the leading cause of the cardiovascular disease (CVD). This study aimed to evaluate the effect of the aqueous extract of *Detarium microcarpum* on metabolic disorders in rats fed with an atherogenic diet. The extract at two doses (200 mg/kg and 400 mg/kg) was co-administered in rats with an atherogenic diet. After 28 days, rats were sacrificed, blood collected in ethylene diamine tetraacetic acid (EDTA) tubes for plasma preparation, and the heart used for homogenate preparation. These plasma and heart homogenates were used to assess lipid profile, cardiac function (NO, ASAT), and hepatic function (ALAT, ASAT, and totals proteins). The results obtained showed that treatment (200 mg/kg and 400 mg/kg extract) led to a significant decrease in TG, VLDL-c, LDL-c, HDL-c, and non-HDL-c compared to untreated rats (positive control) (p < 0.001). Similarly, the cardiovascular risk index (IR, CRR, AC and AIP), were significantly low in the treated groups compared to untreated groups (p < 0.001). Meanwhile, the CPI was higher in threated groups (p < 0.001). The percentage of vascular protection in rats receiving the extract was higher compared to rats treated with atorvastatin. The evaluation of cardiac function showed high levels of NO and ASAT in the heart of rats treated with the extract (p < 0.05). The plasma activity of ASAT and ALAT was low in the groups treated with the extract (p < 0.05). A low plasma level of total protein was also observed in the same groups (p < 0.001). Therefore, the aqueous bark extract of *D. microcarpum* administered orally showed anti-atherogenic, cardioprotective, and hepatoprotective potential.

Keywords: Anti atherogenic, atherogenic diet, cardioprotective, cardiovascular risk, *Detarium microcarpum*, and hepatoprotective

1. Introduction

Cardiovascular disease (CVD) is a category of diseases that involves the heart or blood vessels such as coronary heart disease, myocardial infarction, and angina pectoris, causing deaths in both developed and developing countries (Shafiee-Nick *et al.,* 2017; WHO, 2018). The development and progression of CVD are associated with several risk factors such as dyslipidemia or dyslipoproteinemia, hypertension, hyperglycemia, insulin resistance, obesity, and atherosclerosis (Brunzell *et al.,* 2008). Most cases of CVD complications result from atherosclerosis (Habauzit & Morand, 2012).

Atherosclerosis, which develops mainly in the arteries, is strongly associated with dyslipidemia characterized by high plasma levels of VLDL-c, LDL-c, total cholesterol (TC), non-HDL-c, triglycerides (TG), and low levels of HDL-c. Non-HDL-c represents the major lipoprotein group linked with a higher risk of cardiovascular diseases. Indeed, the increase in plasma cholesterol levels leads to a change in the impermeability of arterial endothelial cells, thus allowing lipids, particularly LDL-c particles, to migrate into the arterial wall (Al-Qahtany *et al.,* 2018). Once in the subendothelial space, these small LDL-c particles will be oxidized causing the endothelial cells to express the adhesion molecules ICAM-1, VCAM-1, E, and P-selectin (Prasad *et al.,* 2014). These adhesion molecules expressed on the endothelial surface will recruit circulating monocytes that will differentiate into macrophages. These macrophages, by expression of the receptors A, B1, CD36, CD68 will massively accumulate cholesterol (Bergheanu, Bodde & Jukema, 2017) and transform into foamy cells. The atherogenic process will continue with a set of vascular modifications. These changes include fat streaks, chronic

inflammation, pathological thickening of the intima, the appearance of fibrous plaques vulnerable to rupture, thrombosis, or stenosis (Aziz & Yadav, 2016). The initiation and evolution of the atherosclerotic process are strongly associated with oxidative stress which is characterized by an increase in the production of reactive oxygen species (ROS). These ROS cause a narrowing of the arterial wall (vasoconstriction) at the vascular level by decreasing the bioavailability of nitric oxide (NO) and the loss of intracellular tissue components (liver, hearts, kidneys, brain) as well as their function by altering their membrane architecture (lipoperoxidation). This damage caused by ROS is due to their toxicity on lipids, proteins, and nucleic acids which are major components of cells (Valko, Rhodes, Moncol, Izakovic & Mazur, 2006). Given the complexity of the mechanisms involved in atherogenesis and the associated complications, the search for new compounds with multiple pathways of action is becoming a necessity.

Polyphenols, especially flavonoids, are compounds derived from plants with variable biological properties. They have beneficial effects on vascular health (inhibition of LDL-c oxidation), dyslipidemia (decrease in LDL-c, TC, TG and increase in HDL-c), inflammation (decrease in pro-inflammatory cytokine production), diabetes (hypoglycemic and insulin sensitizer effect), antioxidant, endothelial dysfunction (inhibits the expression of adhesion molecules, promotes vasodilatation) (Morand & Milenkovic, 2014; Amiot, Riva & Vinet, 2016). For several decades, low-income populations have been using medicinal plants for the management of several pathologies. *Detarium microcarpum* or small sweet detar, a woody plant of the Fabaceae family (Sani, Agunu, Danmalam & Ibrahim, 2014), is one of these plants. In traditional medicine, the decoction or maceration of the leaves, roots, fruits, and bark of this plant is used to treat constipation, meningitis, arthritis, tuberculosis, hypertension, and rheumatism (Sani, Agunu, Danmalam & Ibrahim, 2014; Oibiokpa, Godwin, Abubakar & Kudirat, 2014). The decoction of the stem bark is traditionally used in the treatment of hemorrhoids, blennorrhoea, simple and sanguinolent diarrhea (Adama, 1997). The studies carried out by Hama, Ouedraogo & Adama in 2019, on the methanolic extract of various organs of this plant revealed that the stem bark contained more polyphenols and had a better antioxidant activity *in vitro*. View its antioxidant activity correlated with the polyphenols they contain; this study was carried out to evaluate some biological activities of *Detarium microcarpum* stem bark extract in rats fed with an atherogenic a diet. The effect on lipid profile, atherogenic index, markers of cardiac and hepatic function was evaluated.

2. Methodology

2.1 Reagents

Analytical grade reagents used were purchased from Sigma Aldrich (USA). They were: Folin-ciocalteu reagent, catechin, ethanol, aluminum chloride, potassium acetate, quercetin, sodium chloride, sulfanilamide, naphthyl ethylene diamine dichloride, bovine serum albumin, orthophosphoric acid, L-alanine, L-aspartate, alpha-ketoglutarate, mono, and disodium phosphate.

2.2 Plant Material

The stem bark of *D. microcarpum* was collected in Kousseri (Far North region of Cameroon) and identified at Cameroon national herbarium as No. 49834. These barks were then dried at ambient temperature in a dark room, ground to a powder, and used to prepare the aqueous extract.

2.3 Extraction

The aqueous extract was prepared by decoction. Briefly, 500 g of powder was dissolved in 4 L of distilled water. The extraction ratio was 1/8 (w/v). The mixture was homogenized and then brought to a boil for 45 minutes. The decoction from this boiling was cooled and filtered using Whatman paper No. 1 (Whatman Int. Ltd., Maidstone, U.K). The filtrate obtained was evaporated in the oven at 60 ℃ for 72 hours. The extract obtained was stored at 25 ℃ in the laboratory.

2.4 Quantitative Phytochemical Analysis of the Extract

2.4.1 Determination of Total Polyphenol Content

The amount of total polyphenols in the extract was determined using the Singleton and Rossi method (1965). Briefly, 30 μL of extract (1 mg/mL) was added to 1 mL of the 0.2 N Folin Ciocalteu reagent. A Spectrophotometer (Genesys 20) was used to measure the absorbance at 750 nm of the complex formed between Folin and polyphenols after 30 minutes of incubation at room temperature against the blank. Curcumin (0- 1000 mg/mL) was used as standard and the result obtained was expressed as equivalent mg of curcumin per g of dry matter.

2.4.2 Determination of Total Flavonoid Content

The presence of flavonoids in the extract was assessed according to the procedure described by Aiyegoro & Okoh (2010). For a 1 mL extract (5 mg/mL), 1 mL 10 % aluminum chloride, 1 mL potassium acetate (1 M) and 5.6 mL distilled H_2O was added and mixed. The mixture was then incubated at room temperature for 30 minutes. A spectrophotometer (Genesys 20) was used to measure the absorbance at 420 nm against the blank. The standard was catechin (0 - 40 µg/mL) and the flavonoid concentration was expressed in µg catechin per g of dry material.

2.5 Experimentation

2.5.1 Food Composition

Table 1 shows the composition of the diets used in this study.

Table 1. Dietary composition of the normal and atherogenic diet (Ble-Castillo et al., 2012, with some changes)

Ingredient	Composition in g per 100 g of food	
	Normal diet	**Atherogenic diet (DESF)**
Milk	10	10
Fish meal	10	10
Refined palm oil	10	12.5
Margarine	/	12.5
Sucrose	/	18
Depulped cornflour	35	18
Wheat flour	20	15
Salt	1.5	1.5
Bone meal	2.5	2.5
Polyvitamins	1	1
Fibers (cellulose)	1	1
Distilled water	10	/

DESF: a diet enriched with sucrose and fat

The changes made on the composition of the atherogenic diet were a reduction in the proportion of saturated fats, the addition of unsaturated fats, and an increase in the amount of sucrose.

2.5.2 Animals

Animals were handled according to the European Union Animal Care (CEE Council 86/609) guideline adopted by the Cameroon National Ethics Committee. Adult male *Wistar* albino rats of the age range 12 - 16 weeks and 200-340 g body weight were maintained at their housing conditions with relative humidity (50%) at 25 ℃ temperature. They were obtained from the Laboratory of Nutrition and Nutritional Biochemistry (LNNB) of the University of Yaounde I and were acclimated for 7 days before used. The animals had free access to water, a normal diet, and were maintained in a 12-hour light/dark cycle.

2.5.3 Experimental Design

A total of Twenty-five rats were used. Five of them (210-240 g) constituted the normal group (normal control) and received a normal diet (ND). Twenty (330-340 g) received an atherogenic diet supplemented with fructose at 10 % (g/v). They were divided into four groups of 5 rats as follows: untreated group (positive control): DESG + distilled water; treated group 1: atherogenic diet (DESF) + 200 mg/kg extract; treated group 2: atherogenic diet (DESF) + 400 mg/kg extract and standard group: atherogenic diet (DESF) + atorvastatin at 5 mg/kg. The administration of the extract and atorvastatin was done daily by gavage using an esophageal probe. The experiment lasted 28 days during which the animals had access to water and the diet *ad libitum*. Their weight was taken each week, which allowed the calculation of the weight variation according to the following formula:

$$\text{Weight variation (\%)} = \frac{(\text{Final weight } - \text{Initial weight)\ g}}{\text{Final weight (g)}} \ X\ 100$$

2.5.4 Preparation of Samples

At the end of the experiment, the rats were sacrificed by cervical dislocation after slight ether anesthesia. Blood was immediately collected in the EDTA tubes and centrifuged at 3000 rpm for 10 minutes to obtain the plasma. The heart was then collected for the preparation of the 10% (w/v) homogenate in NaCl (0.9%). Succinctly, the

organ after sampling was rinsed in sodium chloride (0.9%) and then wrung out on filter paper and crushed. The corresponding volume of NaCl was added and centrifuged at 900 g for 10 minutes and the supernatant was recovered. Both samples (supernatant and plasma) were aliquot stored at -20 ℃ for biochemical analyses.

2.6 Determination of Lipid Profile Parameters

Total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-c) were assessed in plasma using standard chronolab brand assay kits. Low-density lipoprotein cholesterol (LDL-c) and very-low-density lipoprotein cholesterol (VLDL-c) were determined using the formula of Friedewald, Levy & Friedrickson (1972).

$$LDL - c \ (mg/dL) = TC - (HDL - c + \frac{TG}{5})$$

$$VLDL - c \ (mg/dL) = \frac{TG}{5}$$

The concentration of non-HDL cholesterol was calculated using the formula established by Brunzell *et al.,* 2008):

$$non\text{-}HDL\text{-}c \ (mg/dL) = \ [TC] - [HDL - c]$$

2.7 Calculation of Insulin Resistance and Atherogenic Indices

Lipid profile parameters were used to calculate insulin resistance, atherogenic indices cardioprotective index, and vascular protection. Insulin resistance (IR) was assessed using the following formula:

$$IR = \frac{[TG]}{[HDL - c]}$$

The atherogenic index of plasma (AIP), atherogenic coefficient (AC), cardiac risk ratio (CRR), and cardioprotective index (CPI) were calculated as follows.

$$AIP = Log \left(\frac{[TG]}{[HDL-c]}\right) \qquad \text{(Althunibat *et al.;* 2019)} \ ;$$

$$AC = \frac{([TC]-[HDL-c])}{[HDL-c]} \qquad \text{(Althunibat *et al.;* 2019)} \ ;$$

$$CRR1 = \frac{[TC]}{[HDL-c]} \ ; \quad CRR2 = \frac{[LDL-c]}{[HDL-c]} \qquad \text{(Ikewuchi \& Ikewuchi, 2009a, b)};$$

$$CPI = \frac{[HDL-c]}{[LDL-c]} \qquad \text{(Oršoli'*et al.,* 2014).}$$

The formula below was used to calculate vascular protection.

$$Vascular \ protection \ (\%) = \frac{AIP \ positive \ control - AIP \ treated \ group}{AIP \ positive \ control} \ x \ 100$$

2.8 Determination of Cardiac Function Parameters

Plasma and cardiac nitric oxide (NO) was evaluated based on the diazotization reaction (Griess, 1879; Chaea, Lee, Kim & Bae, 2004) and the activity of cardiac aspartate aminotransferase (ASAT) by that of Reitman & Frankel (1957).

2.9 Determination of Hepatic Function Parameters

Plasma activity of alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) was evaluated by the colorimetric method described by Reitman & Frankel (1957). The determination of total plasma protein levels was carried out using the method of Lowry, Rosebrough, Farr & Randall (1951).

3. Statistical Analysis

The results obtained were expressed as mean ± standard error on the mean. The values were analyzed with the statistical package for social science (SPSS) software version 20.0. The ANOVA test was used for the descriptive analysis and the comparison between the groups was performed by the post hoc LSD test. The difference in significance was noted at $p < 0.05$ and $p < 0.001$. The excel spreadsheet was been used to plot the graph.

4. Results

4.1 The Polyphenol Content of the Extract

The total polyphenol and flavonoid content of aqueous bark extract of *D. microcarpum* is shown in table 2.

Table 2. Total polyphenol and flavonoid content of the aqueous bark extract of *D. microcarpum*

Detarium microcarpum	**Aqueous bark extract**
Total polyphenols (mg ECu/g of DM)	777.50 ±9.14
Total flavonoids (μg d'EC/g of DM)	53.62 ± 1.63

ECu: Curcumin equivalent, EC: Catechin equivalent; DM: dry material.

4.2 Effect of Oral Administration of Extract on the Body Weight of Rats

An increase in weight was observed in both control groups (Figure 1). However, administration of aqueous bark extract at doses of 200 mg/kg/day and 400 mg/kg/day and atorvastatin at 5 mg/kg/day respectively limited weight gain in the different treated groups. This effect was more pronounced in the group treated with the 200 mg/kg/day extract.
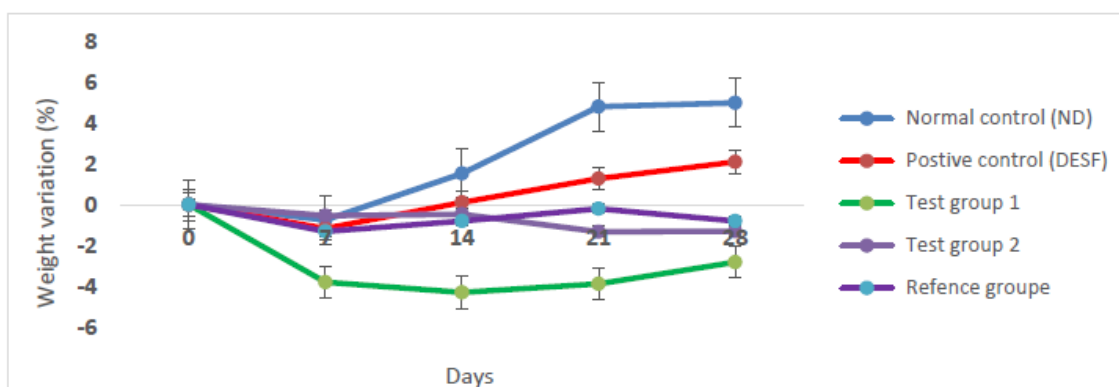

Figure 1. Influence of administration of the aqueous extract of *Detarium microcarpum* bark on the weight variation of experimental rats

n= 5 ; ND : normal diet ; DESF : diet enriched in sugar and fat ; Treated group 1 : DESF + 200 mg/kg/day extract ; Treated group 2 : DESF + 400 mg/kg/day extract ; Standard group : DESF + 5 mg/kg/day atorvastatin

4.3 Effect of Detarium microcarpum Extract on Plasma Lipid Profile

An increase was observed in TG, TC, VLDL-c, non-HDL-c and a decrease in HDL-c in the untreated group (positive control) compared to the normal control group (p < 0.05) (table 3). Oral administration of the extract at doses of 200 mg/kg body weight and 400 mg/kg body weight limited the increase in TG, TC, VLDL-c, LDL-c, and non-HDL-c compared to the untreated group (p < 0.001). The extract at both doses resulted in an increase in HDL-c (p < 0.001).

Table 3. Influence of extract on plasma levels of triglyceride, total cholesterol, very-low-density lipoproteins cholesterol, low-density lipoproteins-cholesterol, high-density lipoproteins-cholesterol (HDL) and non-HDL-c

	Normal control (ND)	Positive control (DESF)	Treated group 1	Treated group 2	Standard group
TG	31.13±1.40[a]	129.90±0.40[b]	77.61±0.60[c*]	85.65±0.04[d*]	62.69±4.48[e*]
TC	65.07±5.30[a]	92.07±0.25[b]	91.92±0.36[b]	92.07±0.60[b]	65.43±4.48[da]
VLDL-c	6.23±0.28[a]	25.98±0.08[b]	15.52±0.12[c*]	17.13±0.01[cd*]	12.53±0.90[e*]
LDL-c	30.31±4.60[a]	49.55±0.41[b]	31.02±0.99[a*]	21.17±0.94[c*]	17.53±1.31[d*]
HDL-c	28.53±0.60[a]	16.54±0.41[b]	45.38±0.99[c*]	53.77±0.94[d*]	35.37±0.91[e*]
non-HDL-c	36.75±4.70[a]	75.53±0.16[b]	46.54±0.63[c*]	38.30±0.34[d*]	30.06±3.56[a*]

n= 5 ; ND : normal diet ; DESF : diet enriched in sugar and fat ; Treated group 1 : DESF + 200 mg/kg/day extract ; Treated group 2 : DESF + 400 mg/kg/day extract ; Standard group : DESF + 5 mg/kg/day atorvastatin ; TG ; triglyceride ; TC : total cholesterol ; VLDL : very low density lipoproteins-cholesterol ; LDL : low density

lipoproteins-cholesterol ; HDL : high density lipoproteins-cholesterol ; c: choleterol ; the assigned values of the alphabetic letters are significantly different (p < 0.05); * represents the difference in significance (p < 0.001) between the positive control and the treated groups.

4.4 Effect of the Extract on Insulin Resistance and Atherogenic Risk

Table 4 presents data on the effects of the extract on IR, CRR, AC, AIP, CPI, and vascular protection (%). IR, CRR, AC and AIP were higher in rats of the positive control compared to the normal control groups (p < 0.001). CPI was low in positive control than normal control groups (p < 0.001). On the other hand, the administration of the extract at two doses and atorvastatin resulted a significant decrease in IR, CRR, AC and AIP, in the treated compared to positive control (p < 0.001). While, the CPI was higher in threated groups (p < 0.001). The percentage of vascular protection obtained was higher in the group treated with 400 mg/kg extract body weight compared to the group treated with 200 mg/kg extract and 5 mg/kg atorvastatin.

Table 4. Effect of the extract on insulin resistance, atherogenic indices, and vascular protection

	Normal control (ND)	Positive control (DESF)	Treated group 1	Treated group 2	Standard group
IR	1.09 ± 0.03^a	7.88 ± 0.17^b	$1.71 \pm 0.02^{c*}$	$1.59 \pm 0.03d^{c*}$	$1.76 \pm 0.08^{ad*}$
CRR_1	2.27 ± 0.14^a	5.58 ± 0.12^b	$2.03 \pm 0.04^{c*}$	$1.70 \pm 0.03^{a*}$	$1.84 \pm 0.08^{c*}$
CRR_2	1.06 ± 0.14^a	2.99 ± 0.03^b	$0.68 \pm 0.01^{c*}$	0.39 ± 0.01^{dc}	$0.49 \pm 0.02^{ec*}$
AC	1.27 ± 0.14^a	4.58 ± 0.12^b	$1.02 \pm 0.04^{c*}$	$0.71 \pm 0.03^{d*}$	$0.84 \pm 0.08^{e*}$
AIP	0.04 ± 0.01^a	0.90 ± 0.01^b	$0.23 \pm 0.01^{a*}$	$0.20 \pm 0.01^{a*}$	$0.24 \pm 0.02^{c*}$
CPI	1.04 ± 0.14^a	0.45 ± 0.01^b	$1.47 \pm 0.01^{c*}$	$2.56 \pm 0.07^{d*}$	$2.06 \pm 0.10^{e*}$
% protection	-	-	74.44	77.78	73.33

n= 5 ; ND : normal diet ; DESF : diet enriched in sugar and fat ; Treated group 1 : DESF + 200 mg/kg/day extract ; Treated group 2 : DESF + 400 mg/kg/day extract ; Standard group : DESF + 5 mg/kg/day atorvastatin ; CRR : cardiac risk ratio ; AC : atherogenic coefficient; AIP : atherogenic index of plasma ; IR : insulin resistance ; CPI : cardiprotective index ; The assigned values of the alphabetic letters are significantly different (p < 0.05) * represents the difference in significance (p < 0.001) between the positive control and the treated groups.

4.5 The Ability of the Extract to Improve Cardiac Function

Plasma nitric oxide (NO) in rats in the groups receiving aqueous bark extract of *D. microcarpum* (treated group) at both doses was significantly lower compared to positive control (p < 0.05) (Table 5). However, in the heart, NO was higher in the treated groups than in the untreated group. The same observation was made after the evaluation of the activity of plasma aspartate aminotransferase (ASAT). In treated groups, there was a significant increase (p < 0.05; p < 0.001) in cardiac NO, ASAT and significant decrease (p < 0.05; p < 0.001) in plasma NO.

Table 5. Effect of the extract on cardiac, plasma nitric oxide levels and cardiac activity of aspartate aminotransferase

	Normal control (ND)	Positive control (DESF)	Treated group 1	Treated group 2	Standard group
NO (Cardiac)	42.72 ± 0.93^a	33.67 ± 0.20^b	$36.15 \pm 0.64^{c*}$	$38.24 \pm 0.44^{d*}$	$37.54 \pm 0.62^{e*}$
NO (Plasma)	32.69 ± 1.94^a	83.56 ± 2.68^b	45.75 ± 0.70^c	$36.94 \pm 0.46^{ad*}$	$23.95 \pm 0.34^{e*}$
ASAT (Cardiac)	49.05 ± 0.39^a	41.66 ± 0.51^b	44.17 ± 0.46^c	43.33 ± 0.47^d	44.05 ± 0.58^e

n= 5 ; ND : normal diet ; DESF : diet enriched in sugar and fat ; Treated group 1 : DESF + 200 mg/kg/day extract ; Treated group 2 : DESF + 400 mg/kg/day extract ; Standard group : DESF + 5 mg/kg/day atorvastatin ; NO : nitric oxide; ASAT: aspartate aminotransferase; the assigned values of the alphabetic letters are significantly different (p < 0.05) ; * represents the difference in significance (p < 0.001) between the positive control and the treated groups.

4.6 The Ability of Extracts to Improve Liver Function

The results of the plasma assessment of transaminase activity (AST and ALT) and total protein levels are presented in Table 6. In the groups treated with the extract, there was a decrease in total proteins compared to the untreated group (p < 0.001). The activity of ALAT and ASAT in the group receiving the extract at 200 mg/kg

body weight was low compared to the untreated group. While the activity of both transaminases was higher in the group treated with 400 mg/kg body weight. In the standard group, these activities were lower compared to the untreated group ($p < 0.001$).

Table 6. Effect of the extract on plasma activity of alanine aminotransferase, aspartate aminotransferase, and total protein levels

	Normal control (ND)	Positive control (DESF)	Treated group 1	Treated group 2	Standard group
ALAT	96.25±2.98[a]	275.73±17.84[b]	263.00±7.72[b]	300.19±40.17[db]	54.13±5.48[c*]
ASAT	80.53±1.91[a]	139.05±16.05[b]	133.31±5.21[cb]	148.70±9.41[db]	75.95±13.50[a*]
Total protein	25.80±2.23[a]	31.44±0.25[b]	21.06±0.28[c*]	22.17±0.39[d*]	29.33±1.30[ae]

n= 5 ; ND : normal diet ; DESF : diet enriched in sugar and fat ; Treated group 1 : DESF + 200 mg/kg/day extract ; Treated group 2 : DESF + 400 mg/kg/day extract ; Standard group : DESF + 5 mg/kg/day atorvastatin ; ALAT : alanine aminotransferase; ASAT : aspartate aminotransferase ; the assigned values of the alphabetic letters are significantly different ($p < 0.05$) * represents the difference in significance ($p < 0.001$) between the positive control and the treated groups.

5. Discussion

Insulin resistance is at the center of several metabolic disorders. These include obesity, type 2 diabetes, dyslipidemia, and non-alcoholic fatty liver disease, all important risk factors for cardiovascular disease (Kato *et al.,* 2015; Oseini & Sanyal, 2016). The objective of this study was to evaluate the effect of the aqueous extract of *Detarium microcarpum* (*D. microcarpum*) bark on the lipid profile, atherogenicity index, markers of cardiac and hepatic function in rats fed on an atherogenic diet (DESF). Preliminary results on the phenolic compound composition of the extract revealed the presence of total polyphenols and flavonoids (Table 2). The values obtained in this work are different from those obtained by Hama, Ouedraogo & Adama (2019) who worked on the antioxidant activity of méthanolic extracts from various organs of *D. microcarpum*. The difference in solubility of polyphenols in the solvent system (Ghedadba, Bousselsela, Hambaba, Benbia & Mouloud, 2014); the environmental conditions of the plant (Falleh *et al.,* 2008), and the extraction method used to justify the variability observed. The existence of these phenolic compounds would suggest that the extract has hypolipidemia, antioxidant, anti-atherogenic, anti-inflammatory potential and will have the ability to improve endothelial function and limit cardiovascular risk. Thus, the preventive effect of *D. microcarpum extract* on certain metabolic and physiological disorders was investigated for 28 days in Wistar rats. The rats fed on a normal diet had a greater weight variation than those receiving DESF alone (figure 1). It was 5.46% and 2.15% for the normal and untreated groups, respectively. This result shows a low dietary intake of untreated rats that would be justified by a decrease in appetite following a change in diet type. However, a high-calorie diet, although poorly consumed, leads to the development of metabolic disorders associated with chronic diseases. Jin, Yi & Mei (2013), showed that rodents fed a high-fat diet develop insulin resistance, dyslipidemia, and hepatic steatosis. Concomitant administration of DESF supplemented with fructose and extract at doses of 200 mg/kg (treated group 1) and 400 mg/kg (treated group 2) and atorvastatin at 5 mg/kg (reference) limited the weight gain of animals in these groups with respective percentage changes of - 2.81%; - 0.97% and -0.80% compared to the untreated group. The polyphenols content in the extract limit rat weight gain by inhibiting lipid digestion, and/or the absorption of their digestion products leading to an increase in their fecal excretion. Polyphenols such as catechin reduce weight gain by stimulating energy expenditure at the cellular level by increasing lipolytic protein expression or by inhibiting lipogenesis through the suppression of fatty acid synthase expression (Yamashita *et al.,* 2007; Kyung, Myoung, Keunae, & Hwang, 2011).

Dyslipoproteinemias are strongly associated with the development of atherosclerosis and its complications (Mulvihill, Burke & Huff, 2016, Bergheanu, Bodde & Jukema, 2017). Thus, all compounds with lipid-lowering and anti-obesogenic properties limit the occurrence of atherosclerosis and its clinical complications like cardiopathy and hepatopathy. In general, the effect of the extract on plasma lipid levels showed a decreased of TG, TC, LDL-c, non-HDL-c, VLDL-c and increased HDL-c compared to the positive control ($p < 0.001$) (table 3). This lipid-lowering potential (especially low levels of LDL-c and non-HDL-c) of the extract reflects its ability to prevent atherogenic risk in rats and associated vascular and tissue complications. The evaluation of the atherogenic index and the IR was therefore correlated with this ability of the extract to lower plasma lipid concentrations. Significant low ($p < 0.001$) IR, CRR, AC, and AIP values were observed in the groups receiving the extract (Table 4), showing its anti-atherogenic potential. This protective power of extract against

atherogenesis induced by DESF is justified by the achievement of high vascular protection and CPI. The indices (IR, CRR, AC, AIP) are useful for assessing the risk of developing CVD, the more accurate the increase, the higher the risk of CVD (Oršoli *et al.,* 2019). On the other hand, the CPI reflects the cardioprotective potential of a compound. The compound is more effective when the index is higher. The presence of polyphenols/flavonoids in *D. microcarpum* extract gives it the ability to normalize plasma lipids, reduce the onset of coronary heart disease, and the accumulation of liver fat as observed. Middleton, Kandaswami & Theoharides (2000), showed that *citrus* flavonoids lowered LDL and TG cholesterol levels in normolipidemic and hyperlipidemic rats. These phenolic compounds used for the prevention/treatment of cardiovascular diseases and their related complications act at various levels. Notably, the inhibition of lipid-digesting enzymes, stimulation of lecithin acyl cholesterol transferase expression (Okafor, Ezeanyika, Nkwonta & Okonkwo, 2015). Flavonoids inhibit the expression of cholesteryl ester transport protein (CETP) which reduces plasma LDL-c levels (Kuivenhoven *et al.,* 1997). This decrease in plasma LDL-c is associated with increased expression of LDL receptors (LDLR) under the control of sterol regulatory element-binding protein-2 (SREBP-2) (Soutar & Naoumova, 2007; You, Su & Zhou, 2008). LDLR is involved in the lysosomal degradation of LDL-c. Flavonoids also lower plasma cholesterol levels by inhibition of acyl CoA cholesterol acyltransferases (ACATs) resulting in inhibition of intestinal cholesterol absorption and hepatic production of VLDL (Keti, Ketan, Randolph, Arroo, Roberta & Matteo, 2017). Studies of Masao *et al.* (2009); Saranan *et al.* (2009) and Nagendra, Rajasekhar & Raghn, (2017) on pine, *Terminalia arjuna*, *Cinnamomum zeylanicum* barks respectively, have also shown beneficial effects on lowering weight gain, preventing dyslipidemia and reducing atherogenicity. Besides, all these barks have shown a protective effect of the aorta against atherosclerotic lesions.

Endothelial dysfunction associated with the overproduction of reactive oxygen species (ROS) is responsible for the onset of atherosclerosis and tissue complications. The key event is vascular production of ROS such as superoxide anion (O^{2-}), catalyzed by NADPH oxidase (NOX) (Griendling, Sorescu, & Ushio-Fukai, 2000). Our results reveal the beneficial effects of *D. microcarpum* on the improvement of endothelial and hepatic function (Table 5 and 6). A decrease in the bioavailability of cardiac NO is indicative of possible vasoconstriction, while an increase in cardiac NO is beneficial for vascular health. Thus, the high level of NO observed in this study is thought to result from the activation of the protein kinase B (Akt) pathway, which by phosphorylation of the endothelial nitric oxide synthase (eNOS) leads to NO production. Rocha *et al* (2019) showed that the leptin/Akt/eNOS signaling pathway is associated with this NO production. Also, polyphenols by inhibition of NOX limit the production of the O^{2-}, which, by action with NO, forms the highly reactive peroxynitrite ion (ONOO-) (Tong, Du-Ok, Bo-Sup, Kyung-Sik & Seong-Gook, 2015). This compound with a high oxidative power leads to an alteration of the tissue membranes. In plasma, the significant low activity of ALAT, ASAT, low level of NO, and total proteins obtained thus reflects the capacity of the extract to protect the liver and heart from oxidative changes associated with a hyperproduction of ROS. Hama, Ouedraogo & Adama (2019) have shown that *D. microcarpum* stem bark can trap free radicals and complex metals. This activity would, therefore, explain the plant's protective power against tissue damage caused by excessive ROS production, as observed in this work.

6. Conclusion

The results of this study revealed the ability of the stem bark aqueous extract of *D. microcarpum* due to the presence of polyphenols to regulate plasma lipid/lipoprotein levels (TC, TG, LDL-c, VLDL-c, HDL-c, and non-HDL-c) and to limit weight gain in rats. *D. microcarpum* significantly reduced atherogenic risk (AIP, CRR, AC), insulin resistance index (IR), and increased cardioprotection index (CPI). In general, this plant improved cardiac and liver function, hence its cardioprotective and hepatoprotective potential. In addition to this work, further studies need to be carried out on transgenic models of cardiovascular disease and monocytes in culture to elucidate the precise mechanism of action of the extract.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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