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Hypolipidemic and antioxidant activity of aqueous extract of *Clerodendrum thomsoniae* Linn. (Verbenaceae) leaves in albino rats, *Rattus norvegicus* (Muridae)

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Abstract
Clerodendron thomsoniae leaves are traditionally used to treat obesity. The effect of Aqueous extract doses (312.5 mg/kg, 625 mg/kg, 1250 mg/kg) on the lipid-lowering and antioxidant potential of *Clerodendron thomsoniae* Linn (*C. thomsoniae*) leaves was assessed. The rats rendered hyperlipidemic after five weeks of feeding with high fat diet were administered aqueous extract of *C. thomsoniae* leaves by oral gavage for four weeks. The total phenolics and the DPPH scavenging activity of the extracts, the lipid profile and the antioxidant parameters of rat plasma administered *C. thomsoniae* extracts were measured. Results revealed that the level of phenolic compounds in the 3 doses (3.1-12.5 mg/L) of extract administered varied from 0.45 (g/100 g D.M.)) to 1.81(g/100 g D.M.). The equivalent variation of DPPH scavenging activities was 78.3% to 97.1%. Biological analysis revealed a decrement in plasma total cholesterol, triglycerides, LDL cholesterol and VLDL cholesterol along with an increase in HDL cholesterol in rats administered *C. thomsoniae* extract as compared to negative control rat group. A dose dependent variation was observed with the highest dose 1250 mg/kg exhibiting the highest percent change 33%, 51%, 37%, 43% and 62%, respectively. Similarly, significant dose-dependent reductions in blood glucose and abdominal fat contents were observed. In addition, the extract induced in a dose dependent manner significant increase in the catalase and superoxide dismutase activities in the plasma, and a decrease in malondialdehyde concentration. Lipid-lowering property of the *C. thomsoniae* extract in chronic hyperlipidemic rats validates its use traditionally as a part of folklore medicine in Ngaoundere-Cameroon, though there is no scientific evaluation to date to our knowledge.

Keywords: Leaves of *Clerodendron thomsoniae* Linn., hypolipidemia, antioxidant

Introduction
Since antiquity, plants have been used medicinally to treat several diseases. According to the World Health Organization (WHO), a variety of drugs are obtained from different medicinal plants and about 80% of the world's developing population depends on traditional medicine for their primary health care needs [1]. Plants are currently used as an ingredient in the preparation of modern medicine. In this sense, several plant species have been explored in recent years and continue to be collected from the wild and play an important role in drug development programs in the pharmaceutical industry [2]. In the specific case of Africa, the flora abounds in several medicinal plants which have already been the subject of several studies [3, 4, 5]. However, other plants such as *Clerodendrum thomsoniae* usually used by traditional healers in Cameroon have not yet been the subject of a real study.
C. thomsoniae is a plant widely distributed in Asia, Australia, Africa and America [6]. Other common names include glory bower, bleeding heart vine, bagfl ower, bleeding glory bower, tropical bleeding heart, and glory tree. In Ngaoundere-Cameroon, this plant is locally called "Poompohga". Ethno-medicinal importance of *C. thomsoniae* has been reported in various indigenous systems of medicines and as folk medicines. The plant is being used as medicines specifically in Indian and Japanese systems of medicine for the treatment of various life-threatening diseases such as syphilis, typhoid, cancer, jaundice and hypertension [6]. In various ancient literature related to healthcare, the powder/paste form and the various extracts of root, stem and leaves are reported to be used as medicine for the treatment of asthma, pyreticosis, cataract, malaria, and diseases of blood, skin and lung [7]. In Cameroon, *C. thomsoniae* is commonly used for the treatment of obesity and diabetes. To prove these ethno-medicinal claims, studies deserve to be conducted extensively on the biological activities of this plant *in vitro* and *in vivo* using various animal models.

In this sense, studies have already been carried out on several plant species of the genus of *Clerodendrum* which are also used for their medicinal properties. Research report revealed that steroids, terpenoids and flavonoids are major active compounds among them [8, 9]. The ethanolic extract of the leaves of *C. infortunatum* Linn showed significant antioxidant activity against DPPH-free radical scavenging activity, reducing power assay and scavenging of hydrogen peroxide [10]. The methanolic extract of leaves of *C. inerme* showed anti-diabetic activity on diabetic rats induced by streptozotocin [11], and significant analgesic activity in acetic acid induced writhing in rats [12].

In the limit of our knowledge, very few if not such studies have been conducted on phytochemical characterization of *Clerodendrum thomsoniae*. In addition, no study has yet been reported on the antioxidant and lipid-lowering properties of aqueous extracts of *C. thomsoniae*. Then, the objectives of the present work were firstly to carry out a phytochemical screening of the aqueous extract of *C. thomsoniae*. Secondly to study *in vivo* the lipid-lowering and antioxidant properties of the aqueous extract *C. thomsoniae.*

Materials and Methods

Sampling and production of *Clerodendrum thomsoniae* powder

Sampling and production of vegetal material powder

Plant material used for this study was obtained from Mbideng, a neighborhood of Ngaoundere, in the Adamawa Region of Cameroon. For the production of *C. thomsoniae* powder, young mature leaves of the plant were carefully cleaned, sorted, graded according to size and dried in a ventilated electric turning dryer (brand Riviera & Bar) at 40 ± 2 °C for 48 h. After drying, the leaves were ground to fine powder using an electric grinder (Culatti, Polymix, France) equipped with a sieve of diameter 500 µm mesh. The obtained powder was directly used for production of the aqueous extract.

Production of aqueous extract of *Clerodendrum thomsoniae*

The powder (2.5 g) was blended with 40 mL distilled water. The different mixtures were placed in a water bath at 70 ± 2 °C and extracted for 30 min under stirring. The mixture was then cooled for 30 min and centrifuged at 1500 g for 15 min at 20 °C using refrigerated centrifuge. The supernatant was collected and the residue was solubilized in 40 mL and re-extracted as mentioned above. The supernatants were combined and concentrated under vacuum in a rotary evaporator and dried in a desiccator at 40 °C. The crude extract was weighed and used to prepare solutions of concentrations 3.12, 6.25 and 12.50 mg/mL representing the 3 tested aqueous extracts of *C. thomsoniae*.

Some chemical analysis of *Clerodendrum thomsoniae* aqueous extract

Qualitative Phytochemical Analysis

Qualitative phytochemical screening was performed in the aqueous extract using standard methods described by Harborne [13] and Bekro *et al.* [14]. Then, for the alkaloid test**,** 2 mL of extract was acidified with a few drops of dilute hydrochloric acid. Then, 1 mL of Dragendorff's reagent was added. The appearance of a reddish orange precipitate was interpreted to indicate the presence of alkaloids. To test for saponins, 9 mL of distilled water was added to 1 mL of extract. The mixture was shaken vigorously for 15 seconds and allowed to stand for 10 minutes. The formation of stable foam (1 cm) was interpreted to indicate the presence of saponins. To identify triterpenoids, 10 mL of chloroform and 1 mL of acetic anhydride was added to 2 mL of the extract. Next, 2 mL of concentrated sulfuric acid was added along the sides of the test tube. The appearance of a red, pink or violet color at the junction indicated the presence of triterpenoids. In order to highlight anthraquinones, 0.5 mL of the aqueous extract was mixed in 5 mL of ether and filtered. The filtrate was then stirred after addition of 5 mL of ammoniac solution. The presence of anthraquinones was confirmed by appearance of a red or violet color.

Quantification of phenols and in vitro antioxidant activity

The total phenolic compound content of the 3 aqueous extracts was determined with Folin–Ciocalteu [15] reagent as described earlier [16] using gallic acid (0.2 g/L) as a standard. The total phenolic content was determined as milligrams of gallic acid equivalent per 100 g of dry matter, using an equation obtained from the standard gallic acid calibration graph. Flavonoid content was determined following the method of Bahorun *et al.*, [17] the amount of flavonoids was calculated from the calibration curve of quercetin standard solutions and expressed as mg quercetin/100 g of dry matter. Tannin level in the extracts was determined by the method of Boham and Kocipai, [18] and results were expressed as mg tannic acid per 100 g of dry matter. The reducing power of the aqueous extract was measured according to the method described by Duh *et al.* [19], and results were expressed in grams of ascorbic acid per 100g of dry matter. DPPH free radical scavenging activity was determined following Brand *et al.* [20], and results were expressed as the percentage decrease of absorbance with respect to the control in which water replaced the aqueous extract.

Animal experiments

Experimental animals

Healthy male Wistar rats (weighing 150 - 200 g) were procured from the animal house of the Faculty of Science, Ngaoundere University, Cameroon. The animals were kept in cages, 1 per cage, with relative humidity (54±2%) in a 12 hrs light/dark cycle at 25 ± 2 °C. They were given access to water and a standard diet ad libitum before experimentation. The experiment was carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Induction of hyperlipidemia

The high fat diet was prepared and administered for 5 weeks except for the normal control group which was fed with standard diet. At the end of 5th week, total cholesterol level in serum was estimated in one rat to verify that the level is greater than 250 mg/dl, the limit value to consider hyperlipidemic as reported by Shinnick *et al.*, [21]. The compositions of the two diet whereas follow:

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% [22].

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% [23].

Experimental design

Hyperlipidemic rats (weighing 320-340 g) were grouped into 6 groups of 5 rats each. Group I was made of normal control rats

fed with standard diet; Group II was negative control group which consisted of hyperlipidemic rats receiving distilled water at a dose of 10mL / kg; Group III, IV and V were tests rats was composed of hyperlipidemic rats administered aqueous extract of *C. thomsoniae* at dose 312.5 mg/kg, 625 mg/kg and 1250 mg/kg, respectively; Group VI was the positive control group made of hyperlipidemic rats treated with standard drug, Atorvastatin (10 mg/kg). The mode of administration of extract was oral, once a day for 4 weeks.

Blood sampling and biochemical analysis

At the end of 28 days' treatment of rats as described above, the rats were overnight-fasted (12 hrs), anaesthetized by inhalation of isoflurane impregnated on a cotton wool and sacrificed. The blood was collected from heart puncture into a vacuum tube and centrifuged at 3000 rpm for 10 min and clear serum was aspirated, stored frozen and then used for desired analysis. Analysis of serum for total cholesterol (TC) [24], triglycerides [25], and high density lipoprotein cholesterol (HDL-c) [26] was performed by using commercial kits and following standard procedures outlined by the producer, Randox Laboratories, UK.

The catalase (CAT) content of serum was determined following the method described by Sinha [27]. The Superoxide Dismutase (SOD) content was determined following the method described by Mishra and Fridovich [28]. The Malondialdehyde (MDA) content was determined following the method described by Yagi [29].

Statistical analysis

The experiments were carried out in triplicate. All data were expressed as mean ± standard deviation and were statistically analyzed using one-way analysis of variance (ANOVA). When statistical differences were found, the Duncan's Multiple Range Test was applied in order to classify variables at the significance level of 5%. The Statgraphics Program (Statically Graphics Educational, version 6.0, 1992, Manugistics, Inc. and Statistical Graphics Corp., USA) was used for the statistical analysis.

Results and Discussion

Chemical properties of aqueous extracts of *C. thomsoniae.*

The results of the phytochemical screening revealing the presence of secondary metabolite compounds in aqueous extracts of *C. thomsoniae* are presented in table 1. Generally aqueous extracts of *C. thomsoniae*. contained alkaloids, saponins, triterpenoids and anthraquinones. The intensity of alkaloids, triterpenoids and anthraquinones doesn't seem to be different in the extract but there is a strong positive test of saponins. These observations may reflect the quantitative difference of these compounds in the extract, then a thorough quantitative analysis of secondary metabolites deserves to be made. Among the most active secondary metabolites of plants, phenolic compounds are an important class. Table 2 illustrates that the aqueous extract of *C. thomsoniae* contains phenolic compounds such as tannins and flavonoids. The content of total phenolic compounds of extract significantly (p<0.05) increased with concentration. The concentration 12.50 g/mL had the highest content (1.81 ± 0.13 g eq gallic acid/100 g DM) of total phenolic compounds while the concentration 3.125g/mL had the lowest (0.55 ± 0.04 g eq gallic acid/100 g DM). Similar increase was observed for tannins which varied from 0.13 ± 0.01 to 0.43 ± 0.02 eq tannic acid/100 g DM). The change in flavonoids contrasted with the other phenols, as no significant difference was observed among the different extract concentrations.

Phenolic compounds play important roles in plant growth and development, particularly in defense mechanisms. Most of the phenolic compounds has potent antioxidant properties, neutralizing the effects of oxidative stress and exhibit ability to chelate heavy metal ions [30]. In this sense, a dose-response relationship was also found in all antioxidant activity effect tests; thus, the antioxidant activities increased (p<0.05) as the concentration increased. The DPPH antioxidant varied from 78.31 ± 0.2% to 97.13 ± 0.54% while the total antioxidant varied from 1.49 ± 0.07 to 1.87 ± 0.02. The antioxidant activities of the extracts were related to the presence of phenolic compounds as reported in the literature [31, 32]. In this vein, positive and significant correlation coefficients were observed between the total phenolic content and reducing power (r=0.83, p<0.05) and DPPH free radical scavenging (r= 0.91, p<0.05). A significant correlation between flavonoid content and reducing power (r= 0.85, p<0.05) and DPPH free radical scavenging (r=0.97, p<0.05) were equally observed. The antioxidant effects of various species of *Clerodendrum* have already been demonstrated [33, 34, 35, 36, 37].

Table 1: Qualitative characteristics of phytochemical composition of aqueous extract of C. *thomsoniae*

Components	Observations	Inference	Intensity
Alkaloids	The resulting solution was turbid	Present	++
Saponins	Persistent foam was observed	Present	+++
Triterpenoids	Pink color was observed	Present	++
Anthraquinones	Red color was observed	Present	++

+++= strong positive test; ++=weak positive test

Table 2: Phenolic compounds (g /100 g dry matter**)** and antioxidant activity of aqueous extract of *C. thomsoniae*

Parameters	Concentration of extracts (mg/mL)		
	3.125	6.25	12.50
Total phenolics	0.55 ± 0.04^a	0.67 ± 0.06^b	1.81 ±0.13^c
Flavonoids	0.37 ± 0.01^a	0.41 ± 0.02^b	0.87 ±.01^c
Tannins	0.13 ± 0.01^a	0.23 ± 0.01^b	0.43 ±.02^c
Reducing power	1.49 ± 0.07^a	1.80 ± 0.01^b	1.87 ±0.02^c
DPPH radical scavenging (%)	78.31 ± 0.2^a	83.80 ± 1.05^b	97.13 ± 0.54^c

Means ±SD (n=3) followed by different letters in the same line are significantly different (p<0.05) as determined by Duncan's multiple range test.

Table 3: Effect of *C. thomsoniae* aqueous extract on Total cholesterol (TC), HDL, Triglycerides (TG), LDL, VLDL and Creatinine in serum of hyperlipidemic rats

Treated groups	Serum parameters (mg/dl)					
	TC	HDL	TG	LDL	VLDL	Creatinine
Normal	62.2±4.3^a	33.3±4.1^a	64.6±2.7^b	20.5±1.5^a	10.5±2.1^a	5.5±0.9^a
Negative C	109.3±11.1^d	30.1±5.2^a	97.6±2.4^d	50.5±2.2^c	19.6±1.5^c	5.9±1^a
Test 312.5	89.5±2.8^c	40.9±2.1^b	75.1±2.2^c	37.9±2.6^b	15.2±1.1^b	5.9±0.9^a
Test 625	90.2±1.2^c	45.7±1.9^c	69.5±2.5^b	34.4±2.2^b	13.8±1.7^b	5.8±1.3^a
Test 1250	73.2±1.9^b	48.9±1.5^d	48±2.2^a	32±2.4^b	11.2±2.4^a	5.6±1.1^a
Positive C	72.5±1.7^b	31.8±4.8^a	46±2.3^a	30±2.5^b	9.2±2.6^a	6.1±0.9^a

Means ±SD (n=3) followed by different letters in the same column are significantly different (p< 0.05) as determined by Duncan's multiple range test. Normal: group of normal rats; Negative C: untreated hyperlipidemia rats received distilled water; Test 312.5: group of rats treated with *C. thomsoniae extract* at dose of 312.5 mg/kg; Test 625: group of rats treated with *C. thomsoniae extract* at dose of 625 mg/kg; Test 1250: group of rats treated with *C. thomsoniae extract* at dose of 1250 mg/kg; Positive C: group of rats treated with atorvastatin at dose of 5 mg/kg.

Effect of *C. thomsoniae* aqueous extracts on lipids profile of hyperlipidemic rats

The consumption of high fat diet is associated with disorders that affect lipid metabolism. Table 4 shows effects of administration of the aqueous extract of *C. thomsoniae* on the lipid profile of hyperlipidemic rats. It can be seen that the untreated hyperlipidemic rats (Group II) showed a significant increase in serum total cholesterol (75.64%), triglyceride (51.15%) and LDL (146.03%) concentration compared to the normal control group rats. These results are in agreement with Ntchapda *et al*., [38] and Marti and Okid [39] who showed that high fat diet is correlated positively with serum total cholesterol and LDL in rats. Administration of *C thomsoniae* extracts to hyperlipidemic rats led to significant reduction of Total cholesterol, LDL and VLDL cholesterol while significant increase of HDL cholesterol was observed. The change in plasma lipid profile was increased as the dose increased. In particular, after treatment with *C. thomsoniae* extract at the dose of 1250 mg/kg, total cholesterol, triglyceride and LDL cholesterol levels decreased from 109.3 to 73.2 mg / dL; 97.6 to 48.0 mg / dL and 50.5 to 32.0 mg/ dL, respectively. The changes expressed in percent equivalent were respectively 33.0%, 50.9% and 36.6%. Fatiha *et al.*, [40], reported that drugs with anticholesterolemic properties are also antioxidant. This suggests aqueous extract of *C. thomsoniae* may have antioxidant properties. This property could justify the use of the decoction of *C. thomsoniae* in traditional medicine to lowering the triglyceride and cholesterol levels in blood [41, 42] then to treat stress. These results are similar with those of several works completed with other plant extracts, such as the aqueous extract of the leaves of *Azadirachta indica* [43] *Cassia occidentalis* [38] in rats subjected to a feeding regime enriched with lipids. Most of these changes may be attributed to phenolic contents which have shown significant negative correlation with the lipids levels. In this respect we observed negative significant linear correlation between total phenolic content of extract and TC (r=-0.51, p<0.01), TG (r=-0.49, p<0.01) and LDL (r=-0.97, p<0.01). This strongly suggests that phenols may contribute in synergy with other compounds of *C. thomsoniae* to lipids lowering in hyperlipidemic rats. We hypothesized that hypolipidemic activity of *C. thomsoniae* extract may be mediated by reducing or inhibiting intestinal cholesterol absorption and increasing reverse cholesterol transport, as shown by other extracts rich in phenolic compounds [44]. Moreover, the presence of saponins in *C. thomsoniae* aqueous extract could also contribute to reduce the lipidemia of the rats. The mechanism of saponins reducing serum total cholesterol may be the formation of insoluble complexes with cholesterol, thus contributing to lower cholesterol levels. Saponins can also combined with the endogenous cholesterol which is discharged by bile, thus preventing re-absorption of cholesterol [45, 46]. Furthermore, we observed an increase in serum HDL levels in the rats treated with *C. thomsoniae* extract. In negative control rat group made of hyperlipidemic rats administered water, the HDL cholesterol value was 30.12 mg/dL. HDL levels increased to 40.89 mg/dL, 45.65 mg/ dL and 48.87 mg / dL in rats treated with extracts at the respective doses of 312.5 mg/ kg, 625 mg/ kg and 1250 mg / kg; Given a maximum increase of 62.25% with the aqueous extract at the dose of 1250 mg/ kg. These results are in agreement with other reports which have noted a significant increase in serum HDL in hyperlipidemic rats treated with plant extracts [44, 38]). Many studies have shown that high HDL levels reduce the risk of cardiovascular disease through their role in eliminating excess cholesterol at the peripheral level towards the liver, which is the main organ of cholesterol metabolism or through their antioxidant, anti-inflammatory, profibrinolytic and anti-thrombotic properties [47].

Table 4: Body weight, food intake, relative weight of the various organs and abdominal fat, and glycaemia of rats

Treated groups	Relative organs weight (%)				Abdominal fat (g)	Glycaemia (mg/dL)	Mean Body weight (g)		Mean Food intake (g)	
	Liver	Heart	Kidney	Testicle			Before treatment	After treatment	Before treatment	During treatment
Normal	3.0±0.1^{a}	0.3±0.1^{a}	0.6±0.2^{a}	1.1±0.1^{a}	1.7±0.3^{a}	51.5±2.5 ^{a}	200.8±4.4^{a}	/	313.6±2.1^{a}	/
Negative C	4,2±0.1^{bc}	0.4±0,1^{ab}	1.0±0.2^{b}	1.7±0.4^{ab}	2.1±0.4^{ab}	83.1±2.3 ^{c}	333.3±6.7^{b}	318.7±4.17^{d}	370.2±4.6^{b}	365.7±3.6^{b}
Test 312.5	3.6±0.8^{abc}	0.3±0.1^{a}	1.0±0.2^{b}	1.5±0.1^{ab}	2.5±0.5^{ab}	64.2±2.5 ^{b}	331.1±5.6^{b}	257.53±4.5^{c}	369.4±4.3^{b}	235.7±4.6^{a}
Test 625	3.4±0.9^{ab}	0.3±0,1^{a}	1.0±0.1^{b}	1.5±0.5^{ab}	2.2±0.5^{ab}	53.5±1.3 ^{a}	334.5±3.8^{b}	230.47±5.4^{b}	362.3±4.7^{b}	238.4±2.5^{a}
Test 1250	3.1±0.3^{a}	0.3±0.1^{a}	0.8±0.1^{ab}	1.5±0.1^{ab}	1.7±0.1^{ab}	51.3±2.6 ^{a}	331.5±7.2^{b}	220.3±3.3^{a}	366.4±4.7^{b}	225.3±6.4^{a}
Positive C	2.9±0.3^{a}	0.3±0.1^{a}	0.8±0.1^{ab}	1.3±0.4^{ab}	1.6±0.2^{ab}	55.5±2.8 ^{a}	326.3±5.8^{b}	237.4±5.6^{b}	370.5±5.8^{b}	241.3±3.5^{a}

Means ±SD (n=3) followed by different letters in the same column are significantly different (p<0.05) as determined by Duncan's multiple range test. Normal: group of normal rats; Negative C: untreated hyperlipidemia rats received distilled water; Test 312.5: group of rats treated with *C. thomsoniae extract* at dose of 312.5 mg/kg; Test 625: group of rats treated with *C. thomsoniae extract* at dose of 625 mg/kg; Test 1250: group of rats treated with *C. thomsoniae extract* at dose of 1250 mg/kg; Positive C: group of rats treated with atorvastatin at dose of 5 mg/kg**.**

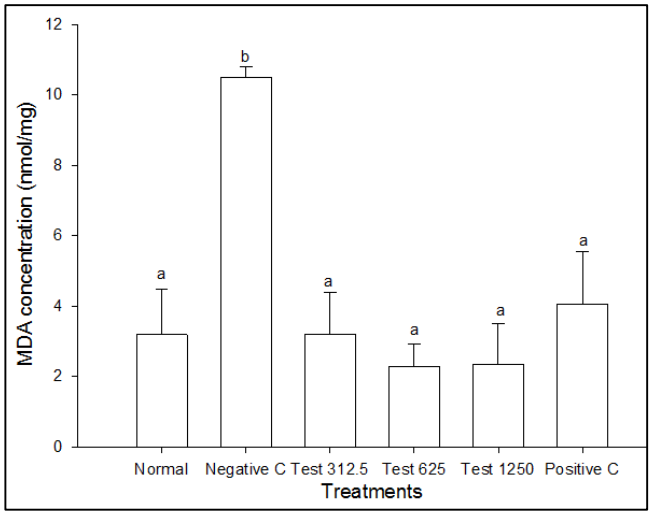
Effect of *C. thomsoniae* aqueous extracts on the oxidative hyperglycemic rats

Hypertriglyceridemia and hypercholesterolemia have been associated with LDL oxidation, protein glycation, glucose auto-oxidation. This leads to an excessive production of lipid peroxidation products which can induce the risk of oxidative stress in subjects with hyperlipidemia [48]. Lipid peroxidation is a condition whereby lipid is oxidized by radicals or reactive oxygen species leading to oxidative stress and cell damage [49]. In absence or reduced capacity of protective mechanisms i.e. antioxidants, pro-oxidant effects predominate leading to development of oxidative stress [50]. This study seems to confirm these previous observations, as there is a significant increase (p<0.05) in the level of serum Malondialdehyde (MDA) in hyperlipidemic rats administered water (10.5 nmol/mg protein) compared to normal rats (3.8 nmol/mg protein) (Figure 1). MDA is a product of lipid peroxidation, its elevation in the blood is an indicator of the presence of reactive oxygen species [51]. MDA is therefore commonly used as an indicator of oxidative stress. Thus, the decrease of the MDA level in the serum reveals the ability of an organism to oxidative stress resistance. The results of our study revealed

that administration of the aqueous extract of *C. thomsoniae* (312.5 mg/kg, 625 mg/kg, 1250 mg/kg) for four weeks significantly decreased ($p<0.05$) serum MDA level compared to the negative control rats, with a percentage decrease of 69.52%, 78.29%, and 77.81% at the respective doses. Negative correlations were observed between serum MDA level and the total phenolic (r=-0.50; $p<0.05$) and flavonoids (r=-0.60; $p<0.05$) contents of the extracts. These correlations are a proof of the involvement of these molecules in the reduction of the MDA levels in animals subjected to fat consumption. Phenolic compounds such as flavonoids are able to reduce reactive oxygen species and thereby reduce the oxidation of lipids and other biological molecules responsible for the formation of MDA [52]. The antioxidant activity of flavonoids depends on the type of phenols, quercetin being one of the most prominent antioxidant, but its level has not been determined in our extract, and this needs to be determined. Our results are similar to those of other plant extracts containing natural antioxidants [53, 54].

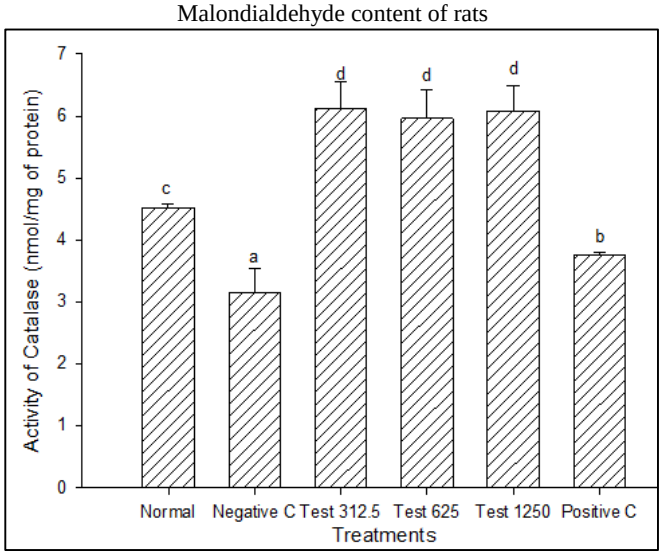
During oxidative stress, tissues respond by inducing enzymatic and non-enzymatic antioxidant defense mechanisms. However, prolonged or enhanced oxidative stress may depress the endogenous antioxidant system by decreasing enzyme activities of SOD, CAT [55] These enzymes work jointly; SOD catalyzes' the dismutation of O_2•- to hydrogen peroxide (H_2O_2), and molecular oxygen (O_2), which is then reduced to water and O_2 by CAT. Figures 2 and 3 shows the influence of administration of aqueous extract of *C. thomsoniae* at different doses and atorvastatin on the plasma concentration of catalase (CAT) and SOD respectively. Plasma CAT and SOD concentrations were significantly ($p<0.05$) higher in the normal control rat group than in the negative control, with values of 4.53 ± 0.03 nmol/mg protein vs. 3.13 ± 0.27nmol/mg for CAT and 0.114 ± 0.01nmol/mg protein vs. 0.067 ± 0.018 nmol/mg for SOD. In addition, plasma CAT and SOD concentrations were significantly ($p<0.05$) greater in all rats administered *C. thomsoniae* than in the normal control, negative and positive control rat groups. Precisely the four-week treatment yielded a high significant ($p<0.01$) increase in CAT at the different doses of the extract (6.13 nmol/mg,5.97 ± 0.32nmol/mg and 6.08 ± 0.403nmol/mg for the respective doses of 312.5, 625 and 1250 mg/kg) against the negative control with a CAT level of 3.14 ± 0.27 nmol/mg. This is equivalent to percentage increases of 35.61%, 32.07%, and 34.51%, respectively. The increase in CAT activity suggests higher H_2O_2 concentrations in peroxisomes [56]. H_2O_2 is the main cellular precursor of the hydroxyl radical which is considered as the most biologically active free radical, hence the removal of H_2O_2 is a good strategy against oxidative stress [57]. Peroxisomal H_2O_2 is an important byproduct of the β-oxidation of fatty acids [58]. Increasing the activities of CAT and SOD enzymes is a characteristic of a good functioning of the body [59]. *C. thomsoniae* exhibits antioxidant effects and in this respect protects the body against oxidative stress. The increase in catalase activity in the hyperlipidemic rats treated with the aqueous extract of *C. thomsoniae* leaves at different doses compared to the negative control shows that the antioxidant enzyme defense could be activated by the bioactive principles present in our extract. Similar observations were reported for some other plant extract such as *Portulaca oleracea* [60].

Figure 3 shows SOD results after treating hyperlipidemic rats for four weeks. A significant difference ($p<0.05$) was observed between the normal group (0.1 ± 0.01 nmol/mg) and the negative control (0.07 ± 0.03nmol/mg). In addition, treatment of hyperlipidemic rats for four weeks with the aqueous *C. thomsoniae* extract at different doses (312.5mg/kg, 625mg/kg, and 1250mg/kg) and atorvastatin (5mg/kg) significantly increased ($p<0.05$) SOD activity in blood plasma compared to the negative control by 169% 67.16% 70.15% and 59.70%, respectively. Worth mentioning is the efficacy of the extract at the dose 312.5 mg/kg compared to the doses 625 and 1250 mg/kg. At these doses, the CAT activities were 0.18 ± 0.084 nmol/mg, 0.112 ± 0.016 nmol/mg and 0.114 ± 0.016 nmol/mg, respectively. These results point out the fact that flavonoids and polyphenols have antioxidant effects. Treatment of rats under oxidative stress with the aqueous *C. thomsoniae* extract reduced plasma oxidative stress by stimulating SOD and CAT activity, probably due to the influence of bioactive compounds, such as the polyphenols.



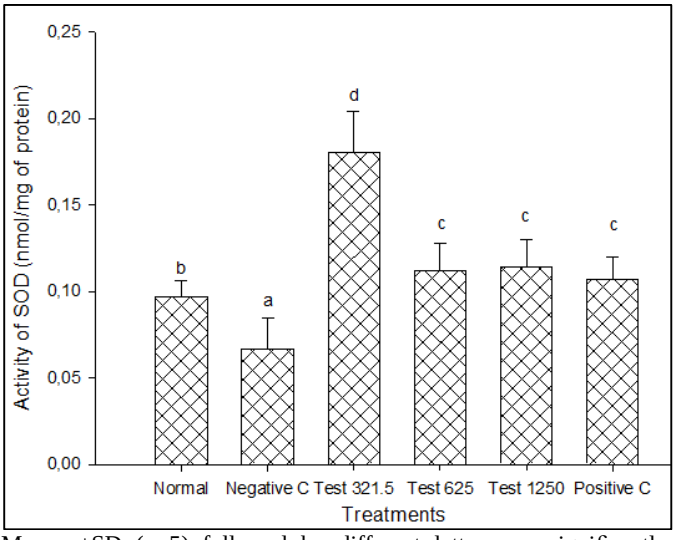
Means ±SD (n=5) followed by different letters are significantly different ($p<0.05$) as determined by Duncan's multiple range test. Normal: group of normal rats; Negative C: untreated hyperlipidemic rats received distilled water; Test 312.5: group of rats treated with *C. thomsoniae extract* at dose of 312.5 mg/kg; Test 625: group of rats treated with *C. thomsoniae extract* at dose of 625 mg/kg; Test 1250: group of rats treated with *C. thomsoniae extract* at dose of 1250 mg/kg; Positive C: group of rats treated with atorvastatin at dose of 5 mg/kg.

Fig 1: Effect of aqueous extract of *C. thomsoniae* on serum Malondialdehyde content of rats



Means ±SD (n=5) followed by different letters are significantly different ($p<0.05$) as determined by Duncan's multiple range test. Normal: group of normal rats; Negative C: untreated hyperlipidemic rats received distilled water; Test 312.5: group of rats treated with *C. thomsoniae extract* at dose of 312.5 mg/kg; Test 625: group of rats treated with *C. thomsoniae extract* at dose of 625 mg/kg; Test 1250: group of rats treated with *C. thomsoniae extract* at dose of 1250 mg/kg; Positive C: group of rats treated with atorvastatin at dose of 5 mg/kg.

Fig 2: Effect of aqueous extract of *C. thomsoniae* on catalase activity



Means ±SD (n=5) followed by different letters are significantly different (p<0.05) as determined by Duncan's multiple range test. Normal: group of normal rats; Negative C: untreated hyperlipidemic rats received distilled water; Test 312.5: group of rats treated with *C. thomsoniae extract* at dose of 312.5 mg/kg; Test 625: group of rats treated with *C. thomsoniae extract* at dose of 625 mg/kg; Test 1250: group of rats treated with *C. thomsoniae extract* at dose of 1250 mg/kg; Positive C: group of rats treated with atorvastatin at dose of 5 mg/kg.

Fig 3: Effect of aqueous extract of *C. thomsoniae* on SOD activity

Effect of *C. thomsoniae* aqueous extracts on organs weights, abdominal fat and glycaemia

The consumption of a high fat diet induced a significant increase in the body weight (35 - 40%) of rats in concordance with previous studies [61, 62]. This could possibly be related to increase of food intake of animals. In fact, hyperlipidemic rats have higher food intake (mean value of 368 g) compared to normal rats (313.6 g) during induction of hyperlipidemia. In addition, a significant and positive correlation (r= 0.82, p<0.05) was observed between food intake and body weight gain of animals during hyperlipidemia induction. As reported recently by Jada *et al.* [63], a diet rich in lipids would stimulate the appetite and thus improve food intake. The increase in the body weight of animals was accompanied by an increase in abdominal fat. As shown in Table 4, compared to normal rats (Group I), hyperlipidemic rats had higher abdominal fat (2.1-2.5 g). This increase in the body weight could be explained by the recruitment of new fat cells via factors which hinder the enzymes of the pyruvate dehydrogenase and the Krebs cycle allowing weight gain [64]. The obesity development would result also resistance to leptin which leads to massive recruitment of adipocytes correlated to the imbalance of the balance energy characterized by storage excessive fat [65]. However, administration of *C. thomsoniae* extract to rats appears to induce a significant decrease (p<0.05) in abdominal fat. The most effective dose was 1250 mg/kg administered to Group V animals. In addition to abdominal fat, weight gain of animals induced by a high fat diet also involved an increase in the mass of some organs of the body [66]. In particular, an increase in liver (70%) weight was observed in hyperlipidemia compared to normal rats. The treatment with *C thomsoniae* extract seems to reestablish the organ weight, in this respect administration of *C. thomsoniae* aqueous extract to hyperlipidemic rats led to significant decrease of liver weight, the decrease in weight being dose-dependent. In this respect negatives correlations were observed between the weight of the liver and the dose of the extracts (r= -0.67; p<0.05) or the total phenolic compounds (r=-0.61; p<0.05). Our results were consistent with other [67, 68] and highlighted the prominent role of phenolic in lowering the body weight, fat mass, and triglycerides through enhancing energy expenditure and fat utilization, and modulating glucose hemostasis [68]. Hyperlipidemia and hyperglycemia are usually associated with the development of type 2 diabetes. This is often a sign of endothelial dysfunction [70]. In this sense, hyperlipidemic rats in group II showed a significant increase in blood glucose compared to normal rats. However, the administration of *C. thomsoniae* extract induced a significant drop (p<0.05) in the blood glucose level of the hyperlipidemic rats of groups III, VI and V. This suggests that this extract may possess hypoglycemic properties that deserve to be investigated.

Conclusion

This study provides evidence of hypolipidemiant and antioxidant effects of aqueous extract of *C. thomsoniae. C thomsoniae* aqueous extract is rich in phenolic and flavonoids. Its consumption induces a reduction in Low Density Lipoprotein cholesterol (LDL-c), triglycerides (TG) and an increase in High Density Lipoprotein cholesterols (HDL-c) in hyperlipidemic rats. It also induces an increase in catalase and superoxide dismutase in rats. These results future the potential use of *C thomsoniae* in the management of metabolic disorder. However, several studies need to be done in order to completely draw conclusion: the hypoglycemic and antioxidant effect of the plant extract on the normal rats, and the toxicity of the extract.

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