



Caralluma dalzielii Ethanolic Extract Prevents High-fat-diet-induced Obesity in Mice

**Dramane Pare^{1*}, Adama Hilou¹, Jotham Yhi-pênê N'DO¹,
Nogma Ernest Sombie¹, Samson Guenne¹ and Noufou Ouedraogo²**

¹Laboratory of Biochemistry and Applied Chemistry (LABIOCA), UFR/SVT, 09 BP 848, Ouagadougou 09, University of Ouagadougou, Ouagadougou, Burkina Faso.

²Research Institute for Health Sciences (IRSS), 03 BP 7192 Ouagadougou 03, Ouagadougou, Burkina Faso.

Authors' contributions

This work was carried out in collaboration between all authors. Authors DP and AH designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JYPN, NES and SG managed the analyses of the study. Author NO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The purpose of this study is to determine the effect of *C. dalzielii* extract on weight, biochemical parameter and antioxidant enzymes of obese Mice induced.

Place and Duration of Study: Laboratory of Biochemistry and Applied Chemistry (LABIOCA), Research Institute for Health Sciences (IRSS).

Background: Obesity is a pathology that occurs as a result of energy imbalance and this metabolic pathology is dramatically increasing in developing countries and it is the cause of many morbidities. *Caralluma dalzielii* is a medicinal plant traditionally used in northern Burkina Faso for weight management. The objective of this study was to determine the anti-obesity potential of the ethanolic extract of *C. dalzielii*.

Methods: The ethanol extract obtained by maceration was administered by gavage to NMRI mice for the determination of toxicity, the effect of the extract on weight gain, food intake and a

*Corresponding author: E-mail: paredra@yahoo.fr;

biochemical parameter of serum. The antioxidant and inhibitory activity of digestive enzymes (pancreatic lipase and trypsin), inflammatory enzymes (lipoxygenase and xanthine oxidase) were also determined.

Results: *Caralluma dalzielii* ethanolic extract has exhibited no toxicity (with an LD50 greater than 3000 mg / kg body weight). It caused a reduction of 7.1% on body weight of the animals treated at 400 mg/kg against an increase of 38.16% in the positive control. Animals in the control group showed a higher concentration of triglyceride and LDL-Cholesterol in serum than those that received the extract. *Caralluma dalzielii* extract has inhibited lipoxygenase at $65.75 \pm 0.05\%$ (at 1 mg/ml) greater than that of gallic acid used as a reference ($54.87 \pm 0.04\%$). It has also shown good inhibition potential on pancreatic lipase, trypsin and DPPH radical.

Conclusion: These results suggest that *Caralluma dalzielii* extract may be a good candidate for the establishment of a phytomedicine in the management of obesity and its complications.

Keywords: *Caralluma dalzielii*; obesity; fat; food intake; lipase; inflammatory enzymes; antioxidant.

1. INTRODUCTION

Obesity is a chronic condition characterised by excess body fat that leads to increased body weight [1]. This pathology has become the fifth leading risk factor for global mortality, nearly three million people die each year. Long regarded as a problem of "developed" countries, it increases dramatically in countries with low or average income for several years [2]. In these developing countries, urbanisation is accompanied by a change in eating habits (more meat products, fats, salt and sweets) and a decline in physical activity (Individual or collective transport more accessible), resulting in an increase in the rate of obesity in these countries. In South Africa, 56% of women and 17% of adolescents are overweight or obese. Fifty-two percent (52%) of Cameroon's population is considered obese. Similar rates are found in Nigeria and Gambia, especially among women [3-4]. This disease is a metabolic disorder caused by environmental factors, genetic predisposition, changing dietary habits [5]. Obesity is considered as a chronic inflammatory state because adipocytes and preadipocytes are sources of proinflammatory cytokine production, including Tumor Necrosis Factor α (TNF- α), Interleukin-1 (IL-1) and Interleukin-6 (IL-6). TNF- α inhibits the activity of the chain polymerisation reaction, thereby increasing the interaction of electrons with oxygen to generate superoxide anion [6]. The increase of these molecules in the obese individuals would be responsible for the increase of the observed oxidative stress [7], obesity is most often associated with cardiovascular diseases (such as hypertension, cardiac insufficiency, stroke), type II diabetes, insulin resistance, dyslipidemia, and many cancers [8].

To treat diseases associated with obesity several strategies are used, such as surgical treatments, pharmaceutical drugs, phytotherapy, etc. Pharmaceutical drugs, most of which are from synthetic origin often have many side effects. That is why in recent years, some pharmaceuticals (Sibutramol, Rimonabant, Isomeride, Ponderal and Xenical) have been withdrawn from the market [9]. Given the low accessibility and non-evident long-term safety of synthetic drugs to treat obesity, the search for new natural molecules is becoming a necessity. In herbal medicine, several species of plants are used against this pathology; namely, plants whose extracts possess activities that inhibit the activity of lipases, adipocyte differentiation, or those which increase thermogenesis and anorexia. In Burkina Faso, many medicinal plants are traditionally used by the population for the treatment of obesity and obesity-linked metabolic diseases [10].

The *Caralluma* genus is characterised by the presence of many pregnane glycosides [11]., these molecules isolated in many plants such as *Caralluma fimbriata*, *Hoodia gordonii* are known for their anorectic and antiobesity activities [12-13].

Ethnobotanical studies have shown that *Caralluma dalzielii* (present in northern Burkina) is used against otitis, asthma, malaria, hernia and urinary retention [14]. This plant is used in the Sahelian regions of West Africa (Nigeria, Senegal) as an antispasmodic and analgesic remedy [15]. In Mali its latex is used to treat wounds, the stems are ground and used as a toning remedy, against heart problems [16]. Studies have shown that ethanol extract of *Caralluma dalzielii* N.E.Br. (Asclepiadaceae)

exhibited good anti-hyperglycaemic activities in diabetic rats [17]. The purpose of this study is to determine the effect of *C. dalzielii* on high-fat-diet-induced obesity in Mice.

2. MATERIALS AND METHODS

2.1 Plant Material

The whole plant of *Caralluma dalzielii* was harvested in Gorom-gorom (Burkina Faso) locality located at 360Km on the Ouagadougou-kaya road (12°29'42.7 N, 1°24'1.2 W) in March 2018. The specie was authenticated by Professor MILLOGO R. Jeanne, a botanist at the UFR / SVT of the University I Professor Joseph Ki Zerbo - Burkina Faso. A herbarium was deposited in the UFR / SVT under identification codes ID-17052. Samples were dried under laboratory conditions away from the sun and then pulverised and stored in freezer bags for different extractions.

2.2 Preparation of Extract

The powder (50 g) of plant material *Caralluma dalzielii* was placed in bottles containing 500 ml of absolute ethanol. The bottles were subjected to mechanical stirring for 24 h at room temperature. The macerated were filtered and then concentrated in an evaporator equipped with a vacuum pump and then evaporated to dryness. These extracts preserved and used for different tests.

2.3 Experimental Animals

MNRI mice aged 5 to 6 weeks were obtained from the Pet Shop of Department of Animal Physiology at University Ouaga I Professor Joseph Ki Zerbo -Burkina Faso. Before the experiment, the animals were acclimatised to laboratory conditions for one week at the Animal Transit Room of Research Institute for Health Sciences (IRSS) Burkina Faso. The animals are placed 12 hours in the light; 12 hours in the dark and they had free access to food and water. All experimental animal protocols had complied with the instructions of the Institutional Animal Ethics Committee (directive 2010/63/EU on the protection of animals used for scientific purposes). Ethical approval code: 2010/63/EU, Date of approval: 20 October 2010. The institutional animal ethical guidelines were strictly observed. All authors hereby declare that "Principles of laboratory animal care were followed, as well as specific national laws where applicable.

2.4 Oral Acute Toxicity Study of the Plant Extract

The toxicity was determined according to the method described by OCDE [18]. The mice were randomised into batches of 6 mice (females). The animals were pre-fasted for 12 hours, then the weight of each mouse was taken, and they received a batch dose of extract. The route of administration of the extracts was oral, Signs of toxicity (writhing, panic, moribund state, death) were noted by batch after 2 h, 24 h, 48 h, 72 h and the animals were kept under observation for two weeks.

2.5 Animals Treatment

The animals were divided into five (5) groups of six (6) mice each. A series of three doses (200, 400, 600 mg/kg body weight) of extract preparation was administered to the different groups of animals, with a negative control group that received only the vehicle (water) plus the standard food and a positive control that received the vehicle and hyperlipidic food. The extracts were administered for a volume not exceeding 200 µl. The animals were placed 12 hours in the light; 12 hours in the dark and they had free access to food and water. They were treated for 30 days [19].

- Group 1: Standard Food
- Group 2: Hyperlipidic Food
- Group 3: Hyperlipidic Food + 200 mg / kg body weight of the extract
- Group 4: Hyperlipidic Food + 400 mg / kg body weight of the extract
- Group 5: Hyperlipidic Food + 600 mg / kg body weight of the extract

2.6 Diet Compositions

Normal diet: Protein (26%), corn starch (50%), sucrose (9%), soybean oil (5%), cellulose (5%), mineral mixture and vitamin (5%).

Hyperlipidic diet: protein (26%), corn starch (15%), sucrose (9%), lard (40%), cellulose (5%), mineral mixture and vitamin (5%).

2.7 Anti-obesity Potential of the Extract

2.7.1 In vitro activities of the extract

2.7.1.1 Inhibition of pancreatic lipase

The activity of pancreatic lipase is performed using p-nitrophenyl butyrate (p-NPB) as a

substrate [20]. Swine pancreatic lipase solutions (1 mg / ml) were prepared in 0.1 mM potassium phosphate buffer (pH 6.0) and the solutions stored at -20°C. To determine the inhibition activity of lipase, extract (final concentration of 100 µg / ml), or Orlistat as a positive control was pre-incubated with porcine pancreatic lipase for 1 h in potassium phosphate buffer (0.1 mM, pH 7.2) at 30°C before assaying for porcine pancreatic lipase activity. The reaction then began following the addition of 0.1 µl pNPB as a substrate, all in a final volume of 100 µl. After incubation at 30°C for 5 min, the quantity of p-nitrophenol released in the reaction was measured at 405 nm using a UV-Visible spectrophotometer. Negative control activity was also examined with and without inhibitor. Inhibitory activity (I) was calculated according to the following formula:

$$\text{Inhibitory activity (I\%)} = 100 - ((B - b) / (A - a) \times 100)$$

Where: A represents the activity without inhibitor, a is the negative control without inhibitor, B is the activity of the inhibitor, and b is the negative control with an inhibitor.

2.7.1.2 Inhibition of trypsin

To measure the inhibitory activity of extract on trypsin, 5 µg of trypsin (from bovine pancreas, Sigma) and 5 µg of total protein extract were combined in a suitable volume of buffer (0.2 M Tris-HCl pH 7, 8) to obtain a total volume of 800 µl of enzyme solution. The trypsin and the extract were incubated for 5 min before the addition of 160 µg of N-α-benzoyl-DL-arginine p-nitroanilide (BAPNA) in 200 µl of substrate buffer (0.05 M Tris-HCl pH 8.2, 0.05 M CaCl₂). The liberated p-nitroanilide was monitored for 25 min at 410 nm using a spectrometer [21]. The percentages of inhibition were calculated by the following equations: Percentage of inhibitors = 1 - (OD Extract / OD Enzyme) × 100

2.7.2 In vivo activities of the extract (Effect of extract on weight and food intake)

The effect of the extract on food intake and weight was evaluated by weighing the animals and the food taken [19].

2.7.2.1 Anorexigenic activity of the extract

The effect of the extract on the quantity of food taken of the treated animals was determined by measuring the total amount of food remaining each day for each batch. Food consumption =

total quantity of food given to the animal - quantity of food remaining.

2.7.2.2 Slimming potential of the extract

The effect of the extract on the weight of the treated animals was determined. During the thirty days of the study, the body weight of each animal was measured every three days using a standard weighing device. The net weight gain was calculated as follows: Net weight gain (W) = final weight (W) - initial weight (W).

2.8 Effect of the Extract on Liver Enzymes and Lipid Profile

On the 30th day, the animals were sacrificed after being anaesthetised with ketamine (150 mg/kg of weight). The blood of the animals were collected by cardiac puncture in dry tubes, centrifuged at 3000 rpm for 5 minutes and the serum was taken to evaluate enzymatic parameters of liver (Aspartate Amino Transferase (AST), Alanine aminotransferase (ALT)) and the rate of lipid indices as cholesterol, total triglycerides, LDLc using kits (Kit Spinreact, Spain; Kit Labkit, Chemelex, S.A(Spain)).

2.9 Antioxidant Potential

2.9.1 Activity of endogenous antioxidant enzymes and liver malondialdehyde

Liver tissues were homogenised in a 0.1 M tris buffer (pH 7.0) and centrifuged at 12,000 × g for 10 min. The supernatant was used for the measurement of liver enzymatic and non-enzymatic antioxidants. Therefore, the antioxidant activity of the extracts was measured by the level of oxidation of lipids, and by measuring the activity of the enzymes involved in the body's defence against the phenomena of oxidation. The level of malondialdehyde in the liver was measured according to the method by Ohkawa [22]; Superoxide dismutase was measured using the standard method [23]; Catalase was measured using a standard protocol given by Beers [24].

2.9.2 Inhibition of the radical DPPH. (2,2-diphenyl-1-picrylhydrazyl)

The anti-radical activity of the ethanolic extract (1 mg/ml) was evaluated by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method [25]. This method is based on the reduction in absorbance at 517 nm of the stable free radical DPPH, in the

presence of a hydrogen radical donor [26], three (03) tests were carried out by mixing 100 µl of the sample and 200 µl of DPPH (20 mg / l in methanol). After 15 minutes of incubation, the absorbance is read at 517 nm against a blank (100 µL of methanol and 200 µL of DPPH) using a spectrophotometer (Epoch 251465, Biotek Instruments, USA). Quercetin and gallic acid were used as reference substances. The antiradical activity was expressed in percent inhibition

2.9.3 Reducing power FRAP (Ferric reducing antioxidant power)

The FRAP method is based on the reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) by the reducing compounds following an electron mono electron transfer [27]. In test tube containing 0.5 ml of extract (1 mg / ml), 1.25 ml of phosphate buffer (0.2 M, pH 6.6) and 1.25 ml of potassium hexacyanoferrate (1% aqueous) were added. The mixture was heated at 50°C in a bain-marie for 30 minutes. After cooling, trichloroacetic acid (1.25 mL, 10%) was added, and the mixture was then centrifuged (2000 rpm for 10 minutes). Three aliquots (125 µl) of the supernatant were transferred into a 96-well microplate to which 125 µl of distilled water and then 25 µl of $FeCl_3$ (0.1% aqueous) were added. The reductive power was evaluated at 700 nm against a standard curve of ascorbic acid using a spectrophotometer (Epoch 251465, Biotek Instruments, USA). The experiment was carried out in triplicate (independent tests), and the reducing activity of the extract was expressed in mmol Equivalent Ascorbic acid per gram of extract (mmol EAA / g extract). Quercetin and gallic acid were used as reference substances.

2.10 Anti-inflammatory Potential

2.10.1 Inhibition of lipoxygenase

The inhibitory activity of extract on lipoxygenase (LOX) was determined by the spectrophotometric method [28]. The reaction medium consisted of a mixture of 100 µL of extract or fraction prepared in borate-methanol buffer (1%) and 400 µL of LOX (167 U mL⁻¹). The mixture was incubated at room temperature for 2 min, and the reaction is initiated by adding 500 µL of the substrate solution (linoleic acid, 250 µM in borate buffer). The kinetics of the reaction are monitored at 234 nm for 2 min. The inhibitory activity expressed as percentage inhibition of lipoxygenase was calculated as follows: % I = (1-B / A) x100

A = activity of the enzyme without inhibitor (Δ abs with enzyme- Δ abs without enzyme)
 B = activity of the enzyme with inhibitor (Δ abs with enzyme- Δ abs without enzyme)

2.10.2 Inhibition of xanthine oxidase (XO)

The inhibitory activity of extract on XO (EC.1.1.3.22) was evaluated according to the method described by Filha [29]. The reaction mixture consists of 50 µl of extract or fraction at the final concentration of 100 µg / ml, 150 µl of phosphate buffer (pH 7.5, 1/15 M) and 50 µl of enzyme solution (0.28 U / mL prepared in the buffer). After preincubation of the mixture at 25°C. for 1 min, the reaction was initiated by adding 250 µl of a substrate solution (0.6 mM) and the absorbance was measured for three minutes. A blank was prepared without extract. The Analyses were carried out in triplicate. Quercetin and gallic acid were used as positive controls. The inhibitory activity of xanthine oxidase (XO), expressed as percent inhibition, is calculated using the formula below:

$$I\% = (1 - V / V_0) \times 100$$

I%: inhibition percentage of the XO; V₀: variation of the absorbance per min of the test without the extract; V: Variation of the absorbance per min of the test with the extract

2.11 Phytochemical Analysis of the Extract

2.11.1 Determination of total phenolics

Total polyphenols were determined using Folin-Ciocalteu reagent [30]. A volume of 125 µL of methanol extracts (100µg/mL) was mixed with 625 µL of Folin- Ciocalteu reagent (0.2 N). After 5 min, 500 µL of aqueous sodium carbonate (Na_2CO_3 , 75 g/l) were added. After 2 h of incubation in the dark at room temperature, the absorbances were measured at 760 nm against a blank (0.5 mL Folin-Ciocalteu reagent + 1 mL Na_2CO_3) by using a spectrophotometer. The experiments were carried out in triplicate. A standard calibration curve was plotted using gallic acid (0-50 µg/mL). The results are expressed in milligrams of gallic acid equivalent per 1 g of dry extract (mg EAG / 1 g).

2.11.2 Determination of total flavonoids

The total flavonoid contents of the extract were determined by the colourimetric method [31]. A

volume of 75 μ l of 2% $AlCl_3$ in pure methanol was mixed with an equal volume of 1 mg/ml extract in methanol. The optical densities were read after 10 min at 415 nm using the spectrophotometer. Quercetin (0-100 mg / L) was used as a standard for the development of the standard curve ($y = 0.0289x + 0.0036$, $R^2 = 0.99$). A mixture of 75 μ l of extract and 75 μ l of methanol without $AlCl_3$ served as a blank. In total, three (3) analyses were performed for extract and the result given was an average of the three readings. The results are expressed in milligrams equivalent quercetin for 1 g of dry extract (mg EQ / 1 g)

2.12 Statistical Analysis

All results were expressed as the mean value of several independent experiments ($n = 3$) \pm standard deviation. For statistical analysis, Graph Pad Prism software (version 5.0) and MS Excel software were used to obtain standard curves and graphs, percentages of inhibition, averages and standard deviations. Anova one way followed by the Tukey test was used to measure the degree of statistical significance of the results. A significant difference was considered for $P < 0.05$.

3. RESULTS

3.1 Oral Toxicity Study of the Extract

C. dalzielii extracts showed no mortality at 3000 mg/kg of body weight (bw) after 72 hours of observation following oral administration of the extract to the animals (Table 1). No sign of toxicity was observed in the wellness parameters during the 14-day observation period.

3.2 Effect of the Extract on Body Weight and Food Intake

Fig. 1 shows the weight variations of the animals during the experiment. Nutrition with hyperlipidic food (40% lipid) caused an increase in the weight of animals compared to control. Oral administration of *C. dalzielii* extract (200 mg / kg, 400 mg / kg, 600 mg / kg bw) showed a weight reduction in animals compared to control and those fed with a hyper lipid diet ($P < 0.05$). The dose at 400 mg/kg body weight showed at the end of the experiment the greatest reduction in weight with an average weight of 2.2 ± 1.6 g (7.1%) against an increase of 12.85 ± 4.8 g

(38.16%) for the batch of animals that received only hyperlipidic food (Fig. 2). As for the variation of the food taken, the negative control lot, the batch that received only the hyperlipidic food and the one receiving the extract at 600 mg / kg of body weight showed a significant increase of the food taken with respectively a difference of consumption between the beginning and end of the experiment of 20.63 g, 27.3 g and 22.4 g. Animals treated at 200 and 400 mg / kg showed little variation in food intake. (1 to 6.9 g) (Fig. 3).

3.3 Effect of the extract on liver enzymes and lipid profile

Fig. 4 shows the effect of *C. dalzielii* extract on total cholesterol, HDL-cholesterol (HDLc), LDL-cholesterol (LDLc) triglycerides. Animals in the control group fed with hyper-lipid and standard food had a high triglyceride concentration compared to groups of animals treated with the plant extract. The group treated with a dose of 200 mg / kg had the lowest total cholesterol concentration. The extract has shown a reduction of triglyceride and LDL cholesterol (bad cholesterol) level, and they induced production of HDLc (Good Cholesterol) level in the animal. There was no significant difference between the different groups for ALT levels, Concerning the concentration of AST, the extracts caused a significant reduction compared to the control group (Fig. 5).

3.4 Effect of the Extract on the Antioxidant Enzymes and MDA

MDA rate in the liver was determined (Fig. 6). *C. dalzielii* extract at 600 mg/kg showed the greatest reduction in MDA (0.06 ± 0.002 nMol/mg) in animals treated against the batch of control animals that received the high-fat diet that gave higher MDA content. The antioxidant enzymes such as SOD and catalase were measured in liver homogenates of animals. The results are shown in Fig. 7. Regarding the effect of the extract on the Catalase activity in treated animals, *C. dalzielii* at 200 and 400 mg/kg gave the best activities with 0.167 ± 0.01 U / mg protein and 0.168 ± 0.06 U / mg protein respectively. Animals with a high lipid diet gave the lowest activity of SOD (0.014 ± 0.001 U / mg protein). The highest activity was obtained in treated animals with *C. dalzielii* extract at 600 mg/kg (0.20 ± 0.03 U / mg protein).

Table 1. Oral acute toxicity study of the plant extract

Plant	dose	mice used	Percent of dead				
			2 h	24 h	48 h	72 h	14 days
<i>C. dalzielii</i>	3000 mg/kg body weight	Six (6)	00	00	00	00	00

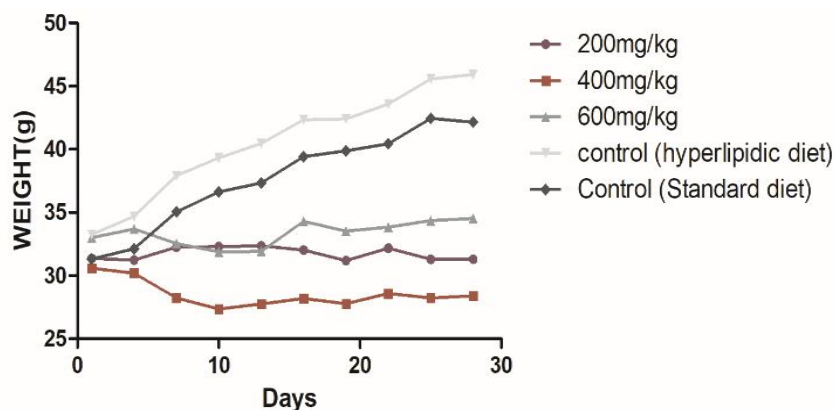


Fig. 1. Effect of *C. dalzielii* extract on weight

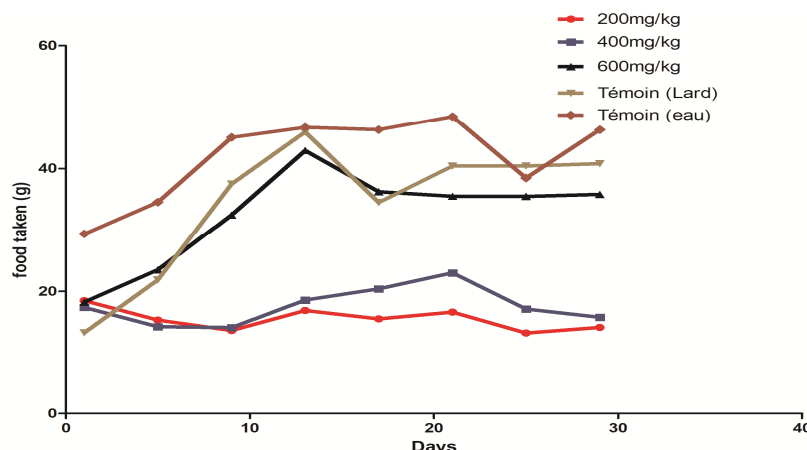


Fig. 2. Effect of *C. dalzielii* extract on food intake

3.5 *In vitro* Enzymatic Activities of the Extract

The ethanolic extract of *C. dalzielii* showed an inhibition of $64.58 \pm 0.86\%$ on the pancreatic lipase at $100 \mu\text{g} / \text{ml}$ of the extract against $90.1 \pm 2.27\%$ for the orlistat used as a reference substance (Figure 8). For the activity on trypsin, the extract inhibited at $31.66 \pm 2.88\%$ for a concentration of $100 \mu\text{g} / \text{ml}$ of extract. Regarding the inflammatory enzyme inhibition test, *Caralluma dalzielii* inhibit lipoxygenase at $65.75 \pm 0.05\%$ at 1 mg/ml greater than that of gallic acid used as reference which gave an inhibition of $54.87 \pm 0.04\%$; against xanthine oxidase at $100 \mu\text{g} / \text{ml}$ the extract showed an

activity of $80.55 \pm 8.41\%$ against $90.90 \pm 00\%$ for quercetin used as reference substances (Fig. 7).

3.6 Antioxidant Activity and Phytochemical Composition of the Extract

The ethanolic extract of *Caralluma dalzielii* neutralise the DPPH radical at $62.727 \pm 2.4\%$ and presented a reduction of the Fe^{3+} ion to Fe^{2+} of $1.51 \pm 0.03 \text{ mmol EAA} / \text{g}$ of extract with a concentration of 1 mg/ml . The determination of total polyphenols and total flavonoids in the extract gave of $368.875 \pm 16.2 \text{ mg EAG} / \text{l g}$ and $9.58 \pm 0.35 \text{ mg EQ} / \text{l g}$ (Table 2) respectively.

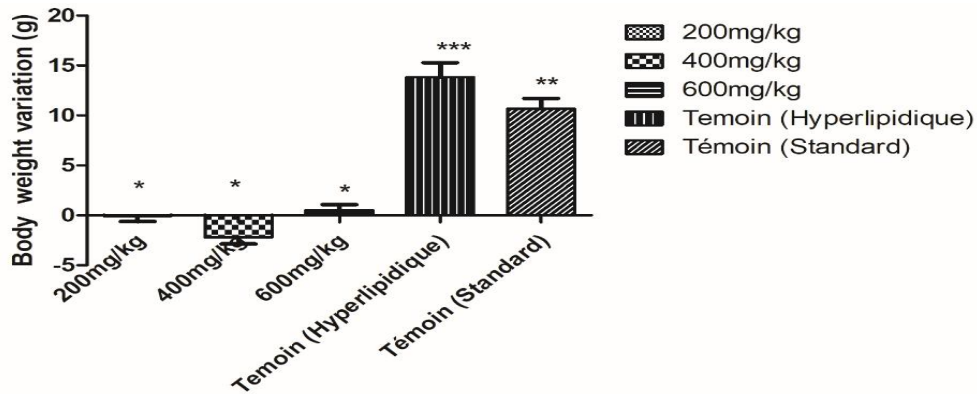


Fig. 3. Effect of the extract on weight variation
 ***P-value is significant at $p < 0.05$

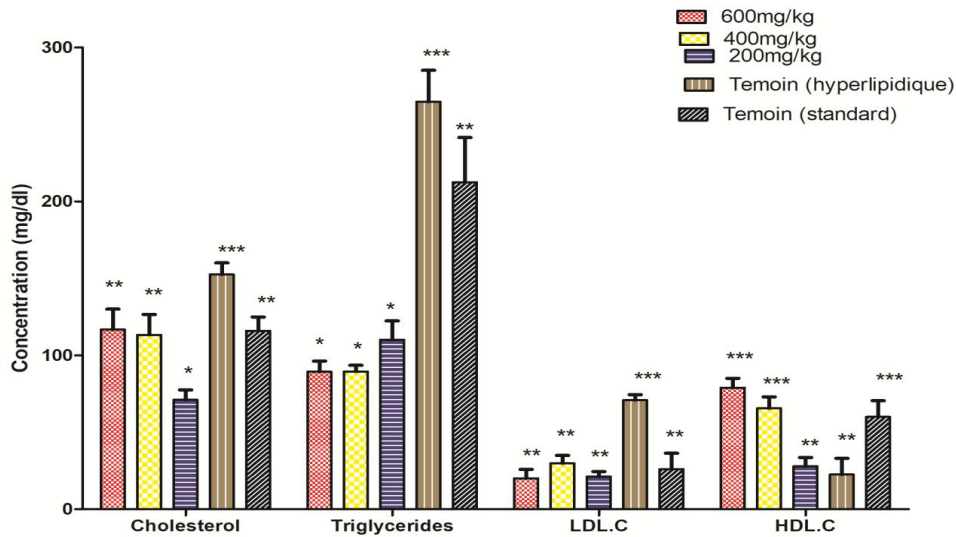


Fig. 4. Effects of the extract on lipid profiles of serum
 ***P-value is significant at $p < 0.05$

4. DISCUSSION

In this study, the anti-obesity activity of *C. dalzielii* extract was determined using hyperlipid food on animal models. Plants generally induce

weight loss through several mechanisms, they can increase satiety [32], increase thermogenesis [33], prevent differentiation of preadipocytes [23] and lipase inhibition [34].

Table 2. Antioxidant activity and phytochemical composition of the extract

Activities		<i>C. dalzielii</i>	Gallic acid	Quercétin
Antioxidant activity	DPPH	62.727 ± 2,4 **	86,63±0,08 ***	82.17 ± 0.30***
	FRAP	1.51 ± 0.03*	8.84± 0.45 ***	4.69± 0.05 **
	Total flavonoids content	9.58 ± 0.35	ND	ND
Phytochemical composition	(mgEQ/1 g)			
	Total polyphenol content (mgEAG/1 g)	368,875 ± 16.2	ND	ND

mgEAG/1 g = milligrams of gallic acid equivalent per 1 g of dry extract; mgEQ/1 g = milligrams equivalent quercetin for 1 g of dry extract; FRAP = Ferric Reducing Antioxidant Power, DPPH = (2,2-diphenyl-1-picrylhydrazyl). ***P-value is significant at $p < 0.05$; Mean ± S.E.M = Mean values ± Standard error of means of three experiments. ND= Non-determined

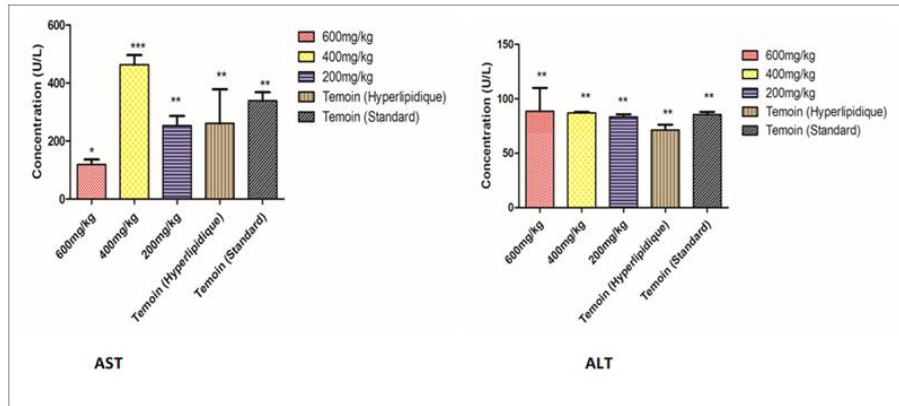


Fig. 5. Hepatic biochemical parameters to liver enzymes
 AST = Aspartate Amino Transferase; ALT= Alanine aminotransferase
 ***P-value is significant at $p < 0.05$

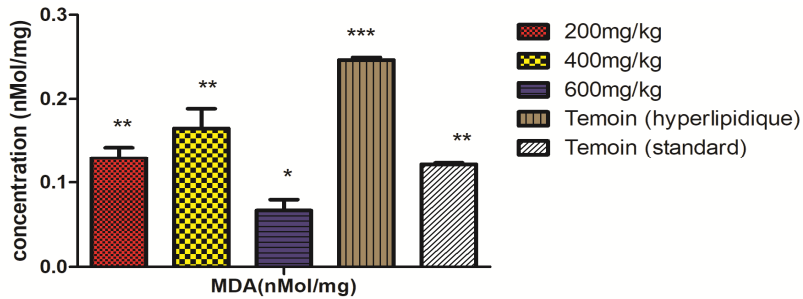


Fig. 6. Effect of extracts on MDA in the liver
 MDA= malondialdehyde, ***P-value is significant at $p < 0.05$; Mean \pm S.E.M = Mean values \pm Standard error of means of three experiments

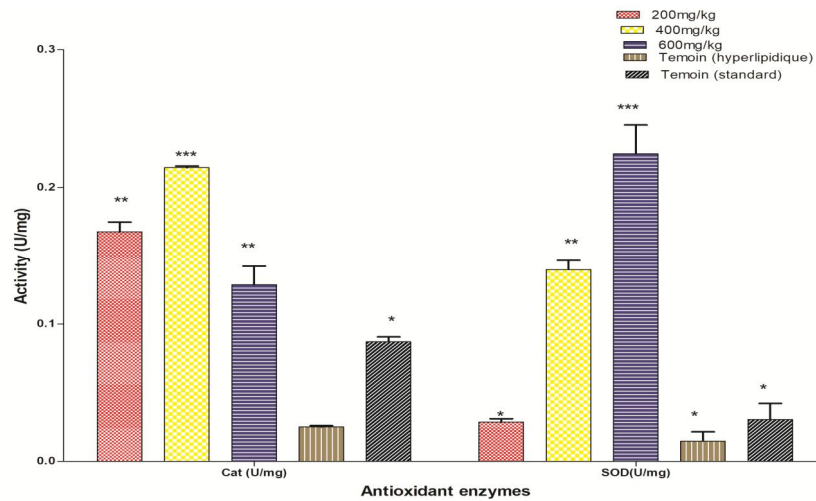


Fig. 7. Effect of extracts on the antioxidant enzymes of the liver
 Cat= Catalase; SOD= Superoxide dismutase
 ***P-value is significant at $p < 0.05$; Mean \pm S.E.M = Mean values \pm Standard error of means of three experiments

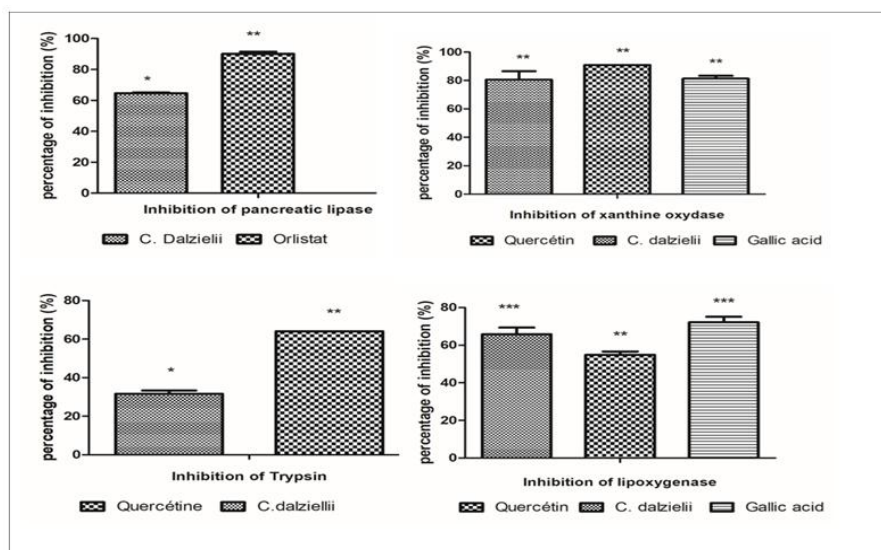


Fig. 8. In vitro activity of *Caralluma dalzielii* extract

***P-value is significant at $p < 0.05$; Mean \pm S.E.M = Mean values \pm Standard error of means of three experiments

Oral administration of *C. dalzielii* extract resulted in weight reduction in the treated animals compared to untreated animals. The extract also has an anorectic effect at a dose of 400 and 200 mg/kg body weight. The ability of the plant extract to reduce food intake coupled with decreased weight gain in treated animals compared to their counterparts what received water (control groups), suggests that *C. dalzielii* could be a good satiety inducer and weight management modulator. The *Caralluma* genus is characterised by the presence of many pregnane glycosides [11]., these molecules isolated in many plants like *Caralluma fimbriata*, *Hoodia gordonii* is known for their anorectic activity, anti-obesity [12-13]. The pregnant pregnans glycosides have indeed isolated from the whole plant of *Caralluma dalzielii* [15], it could, therefore, act on obesity by its anorectic effect. Obesity is a pro-inflammatory condition due to macrophages infiltrating in the adipose tissue, cytokines (produced by macrophages) and adipokines produced by fat cells alter lipid metabolism [35]. Proinflammatory cytokines, tumour necrosis factor α (TNF- α) and interleukin-1 (IL-1) stimulate lipolysis in adipocytes by increasing circulating free fatty acid levels and the production of lipoproteins rich in triglyceride, which will contribute to the increase of serum triglycerides in obese patients. Dyslipidemia characterised by an increase in triglyceride levels with the growth of LDL, cholesterol [36]; which is a major risk factor for cardiovascular diseases.

C. dalzielii in the present study in addition to weight reduction by the three doses caused a significant decrease in triglyceride levels and a lower LDLc level than the control lot made obese with hyperlipidic food (Fig. 4). Our extracts could prevent cardiovascular disease by preventing the accumulation of triglycerides and LDLc in the body. This could explain the traditional use of this plant against heart problems in Mali [37].

The extracts caused a reduction in serum AST and had no effect on ALT levels. Elevation of transaminases is significantly associated with overweight, suggesting that a high proportion of obese individuals have steatosis or non-alcoholic steatohepatitis [38]. The hyperlipidic diet caused in the untreated animals an increase in the AST concentration, which is corrected by the extracts. Oxidative stress is characterized by the Reactive Oxygen Species (ROS) that induce lipid peroxidation, MDA is a secondary product generated during the oxidation of poly-unsaturated fatty acids [39]. The hyperlipidic diet resulted in an increase in MDA in untreated animals compared to those treated that gave a lower MDA concentration. Weight gain was accompanied by an increase in MDA; the concentration of MDA is correlated with weight in obese children and adolescents [40]. In our study, the extract showed an increase in activities of SOD and catalase in the presence of a hyperlipidic diet but showed a decrease in the obese animals. In our study, the extract showed

induction of SOD and catalase synthesis in the presence of a hyperlipidic diet, but these decreased in obese animals. These two enzymes play an important role in the metabolism of reactive oxygen species. SOD is involved in the destruction of the superoxide anion (O_2^-), and its production decreases with increasing body weight [41]. Catalase catalyses the degradation of hydrogen peroxide (H_2O_2) and thus protects the body from the oxidative and harmful action of hydrogen peroxide. This result probably explains the protective effect of *C. dalzielii* extract against oxidative stress in the liver of the mice. The damage to the cell by the oxidant products in obesity is attenuated by the increase in antioxidant enzymes such as SOD, CAT and a reduction in MDA production.

The inhibition of digestive enzymes is a promising alternative for the treatment of obesity, mainly because they act on the small intestine, without acting on the central nervous system, where the usual anorectic agents act. Pancreatic lipase is a key enzyme in lipid digestion for the absorption of triglycerides in the small intestine. It is secreted by the pancreas and hydrolyses triglycerides into glycerol and free fatty acids [42]. This inhibition might be a good way to reduce lipid absorption. Trypsin is an enzyme of the pancreas. It is synthesised as a trypsinogen (inactive proenzyme) and stored in the enzymatic vesicles of acinar cells, from which it is excreted at the time of digestion. When trypsin inhibitors are present in a diet, they can lead to a reduction in the rate of growth in animals due to a decrease indigestible proteins, leading to weight loss and endogenous catabolism of proteins [43].

The extract of *C. dalzielii* showed a good inhibition of the digestive enzymes but lower than the reference molecules used. This activity observed could be due to the presence of polyphenols and flavonoids found in our extract. Indeed, a good number of natural antihyperlipidemic molecules have already been isolated from some plants; Epigallocatechin-3-gallate, kaempferol and quercetin have been shown to inhibit pancreatic lipase [44]. Trypsin inhibitors isolated from *Tamarindus indica* L. seeds showed a reduction in weight and an increase in Cholecystokinin (CCK) levels in the treated animals [45]. CCK is an anorectic hormone that sends satiety signals to the brain. Obesity is associated with an increase in the reactive oxygen species (ROS), this is probably due to the presence of excess adipose tissue itself since adipocytes and preadipocytes have

been identified as a source of pro-cytokines. therefore, an increase of cytokine concentration could be responsible for an increase in ROS [7]. The extract of *C. dalzielii* showed good antioxidant activity by inhibiting the DPPH radical and reducing the ferric ion to ferrous ion. It has also shown good potential to inhibit inflammatory enzymes such as xanthine oxidase and lipoxygenase (LOX). A glycoside isolated in the extract *Conyza bonariensis* have shown an inhibitory activity on xanthine oxidase [46]. Glycosides inhibitory of LOX are isolated in *Calendula officinalis* L. [47]. The inhibitory potentials observed in the extract could be due to the glycosides contained in these species [48-49]. The extract contains polyphenols and flavonoids. These molecules indeed have antiobesity activities and would help reduce weight by their anti-lipase activity [44]. Rutin, quercetin, narreginine inhibit the activity of glycerol-3-phosphate dehydrogenase (GPDH) and the expression of PPAR γ , C / EBPR genes involved in the differentiation of adipocytes. Adipocytes are the cells that store lipids in the body [50]. The presence of flavonoids in the extract might have acted on the fat cells for weight reduction.

5. CONCLUSION

In conclusion, this study indicates that *C. dalzielii* extract appears to be a good and promising agent for the search for molecules capable of modulating obesity because of its potential in inhibiting digestive enzymes and its anorectic effect. They also showed a good reduction of triglycerides and LDLc levels in treated animals indicating its potential as a weight reducing agent.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experimental animal protocols had complied with the instructions of the Institutional Animal Ethics Committee (directive 2010/63/EU on protection of animals used for scientific purposes). Ethical approval code: 2010/63/EU, Date of approval: 20 October 2010. The institutional animal ethical guidelines were strictly observed. All authors hereby declare that "Principles of laboratory animal care were followed, as well as specific national laws where applicable

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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