

## Evaluation of the preventive effect of the total aqueous extract of *Lippia multiflora* Moldenke leaves on anthropometric parameters in rats made obese by a hypercaloric diet

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### ABSTRACT

**Background :** Obesity has become a serious public health problem, with modern treatment being lengthy and costly, and therefore often inaccessible to all patients. Traditional medicine therefore offers an alternative for low-income populations.

**Objectives :** The objective of this study was to evaluate the effect of *Lippia multiflora* aqueous extract on obesity prevention through anthropometric parameters.

**Methods:** We therefore administered the aqueous extract of this plant at doses of 200 and 400 mg/kg bw to rats made obese by a high-calorie diet composed of sucrose and paraffin oil for 28 days.

**Results:** The extract at these different doses showed a decrease in anthropometric parameters (body mass, weight gain, body mass index, and abdominal circumference). This decrease was significant for body mass, weight gain, and abdominal circumference. The extract also restored the relative masses of organs (liver, heart, kidneys, visceral adipose tissue, and abdominal subcutaneous tissue) disrupted by feeding a high-calorie diet in rats at doses of 200 and 400 mg/kg bw.

**Conclusion :** In summary, *Lippia multiflora* possesses anti-obesity effects, allowing it to prevent the onset of obesity due to a high-calorie diet.

**KEYWORDS:** *Lippia multiflora*, Obesity, rats, anthropometric parameters, relative weights

### 1. INTRODUCTION

Obesity is generally due to excessive eating, leading to poor glucose utilization by the body as evidenced by chronic hyperglycemia (1). Oxidative stress is often the cause of obesity (2). Widespread in all continents, overweight, particularly obesity, has become one of the leading causes of mortality worldwide and the leading risk factor for disability (3). Indeed, according to the World Health Organization (4), approximately 2.5 billion adults aged 18 and over were overweight and approximately 890 million obese people were observed in 2022. This value represents 43% of the adult population worldwide. Africa is also facing a growing problem of overweight, especially among children. In 2019, the continent was home to 24% of overweight children under 5 years old worldwide (4). Studies by (5) in the school environment in Côte d'Ivoire showed a prevalence of 5%. Furthermore, there were 4% overweight subjects, 39% underweight, 25% thin and normal subjects were 27%. Obesity was more common in girls (6.8%) than in boys (1.8%). Obesity management is mainly based on a change in nutritional habits (low fat, sugar and sodium), regular exercise and drug treatment. The latter is reserved for the most severe forms of obesity associated with complications (6). Currently, many conventional medications are used for the treatment of obesity. The ineffectiveness of conventional treatments, their adverse effects, and their tolerability issues have led African populations to turn to traditional medicine (7,8).

Plants are considered an important source of raw materials for the discovery of new molecules needed for future drug development.

Today, it is clear that traditional medicine is increasingly used by populations to treat several pathologies such as obesity. It is in this context that we chose *Lippia multiflora*, a plant from the Verbenaceae family commonly used in Ivory Coast in the treatment of obesity. The main objective of this study is therefore to evaluate the preventive potential of an aqueous extract of *Lippia multiflora* on anthropometric parameters in an experimental model of obesity induced orally by a high-calorie diet in Wistar rats.

## 2. MATERIAL

### Plant Material

The plant material consisted of the leaves of *Lippia multiflora* Moldenke (Verbenaceae). They were harvested in Daloa (central-west region of Côte d'Ivoire) during the semi-rainy season in 2025.

### Animal Material

The experiments involved albino rats of the species *Ratus norvegicus*, of the Wistar strain. They were eight (8) to twelve (12) weeks old and weighed between 154 and 218 g. These animals, from the animal facility of the Faculty of Biosciences at Félix Houphouet Boigny University (Cocody, Abidjan), were acclimated to the Animal Physiology Laboratory (Félix Houphouet Boigny University) where the experiments took place. They were kept under standard conditions: 12 hours of light and 12 hours of darkness, at room temperature, with free access to food and water.

## 3. METHODS

### Extraction of the Total Aqueous Extract of *Lippia Multiflora* Leaves

One hundred grams (100 g) of *Lippia Multiflora* leaf powder are decocted in one liter of distilled water. After mixing with magnetic stirring, the decoction is filtered twice (using cotton wool and Wattmann paper). The filtrate is evaporated at 65°C using a rotary evaporator and dried at 45°C in an oven. The method used is that of [9].

### Preventive Effect of *Lippia Multiflora* Leaf Aqueous Extract on Anthropometric Parameters in Obesity Induced by a High-Calorie Diet in Rats

We implemented this method based on the studies of (10). The goal was to preventatively treat a metabolic disorder caused by a high-calorie diet composed of 30% sucrose and refined palm oil.

We divided the rats into five (5) batches of six (6) rats each and then treated them by gavage (oral route) with different products:

- Batch 1: Rats given distilled water at a dose of 5 mL/kg bw;
- Batch 2: Rats given distilled water (5 mL/kg bw) + the solution (30% sucrose at 10 mL/kg bw + refined palm oil at 10 mL/kg bw);
- Batch 3: Rats given metformin (Glyferon\*) at a dose of 0.1 mg/kg bw + the solution (30% sucrose at 10 mL/kg bw + refined palm oil at 10 mL/kg bw);
- Batches 4 and 5 (tested batches): Rats receiving the aqueous extract at doses of 200 and 400 mg/kg bw + the solution (30% sucrose at 10 mL/kg bw + refined palm oil at 10 mL/kg bw).

The rats were subjected to this treatment for 28 days during which the animals' body weights were measured at the end of each week.

### Determination of anthropometric parameters

Anthropometric parameters were determined using the method described by (11). Throughout the experiment, body mass was weighed, as well as abdominal circumference (AC) and body length (naso-anal length) were measured in all rats. These measurements were taken at the beginning and weekly during treatment until the end of the experiment (28 days).

Body mass was measured using a digital scale (Denver Instrument S-234, Germany). Abdominal circumference and naso-anal length were measured manually using a tape measure. Abdominal circumference was measured across the widest area of the rat's abdomen.

In addition, body mass and naso-anal length were used to determine BMI (Body Mass Index) using the following formula:

$$\text{BMI} = \text{Body mass (g)} / (\text{Naso-anal length})^2 (\text{cm}^2)$$

### Organ Harvesting

At the end of the experiments, all rats were weighed and sacrificed according to good laboratory practices. After anesthesia with ether solution, organs such as the liver, heart, kidneys, and adipose tissue were removed with small dissecting forceps and rinsed with 0.9% NaCl solution. The harvested organs were weighed using a precision balance to calculate the relative mass using the following formula (12).

$$\text{Relative mass (\%)} = \frac{\text{Absolute mass of the organ (g)}}{\text{Body mass of the animal on the day of sacrifice}} \times 100$$

#### **Statistical Analysis**

Data were expressed as the mean followed by the standard error of the mean ( $M \pm SEM$ ). Analysis of variance (ANOVA 1) was applied to the results to assess the effects of the different treatments at the 5% statistical significance level. This multiple comparison test was used to compare means. Graph Pad Prism 8.0 software (San Diego, California, USA) was used for statistical analysis.

## **4. RESULTS**

### **Yield of the total aqueous extract of *Lippia multiflora* leaves**

The mass of 100 g of fine powdered *Lippia multiflora* leaves yielded 20.4 g of total aqueous extract of *Lippia multiflora* leaves. Thus, the extraction yield obtained during this preparation is 20.4%.

### **Effect of the extract on anthropometric parameters in rats**

#### **Effect of the extract on body mass in rats**

The body weight of the rats increased in all groups. In batch 1 (distilled water), the increase in body weight was moderate. In contrast, in batch 2, treated only with the sucrose solution and paraffin oil, a rapid increase in body weight was observed, reaching  $236 \pm 4.3$  g. In batches 4 and 5 treated respectively with the extract 200 mg/kg + sucrose solution and paraffin oil and 400 mg/kg + sucrose solution and paraffin oil, a slowdown in the progression of body weight is observed. Body weights of  $198.2 \pm 5.1$  and  $195.2 \pm 4.6$  g are obtained on the 28th day for the doses of 200 and 400 mg/kg bw respectively. The same kinetics of action is noted with  $191.1 \pm 4.3$  g in batch 3 treated with Glyferon\* + sucrose solution and paraffin oil.

**Table 1:** Influence of the extract on the evolution of body weight of rats.

<b>Body weight (g)</b>	<b>Batch 1</b>	<b>Batch 2</b>	<b>Batch 3</b>	<b>Batch 4</b>	<b>Batch 5</b>
<b>Day 0</b>	$174.1 \pm 3.2$	$175.1 \pm 4.2$	$173.2 \pm 3.5$	$176.4 \pm 3.7$	$173 \pm 3.4$
<b>Day 7</b>	$176.6 \pm 3.2$	$189.2 \pm 6.2$	$175.2 \pm 3.5$	$177.2 \pm 4.2$	$175.4 \pm 5.2$
<b>Day 14</b>	$180.2 \pm 4.2$	$210.1 \pm 4.3$	$178.3 \pm 3.5$	$179 \pm 3.76$	$178.6 \pm 4.6$
<b>Day 21</b>	$183.3 \pm 5.2$	$223.2 \pm 4.6$	$183.2 \pm 3.5$	$190.5 \pm 4.6$	$185.4 \pm 4.6$
<b>Day 28</b>	$191.4 \pm 5.1$	$236 \pm 4.3$	$191.1 \pm 4.3$	$198.2 \pm 5.1$	$195.2 \pm 4.6$

**Batch 1:** control on normal diet; **Batch 2:** control on diet with sucrose solution and paraffin oil; **Batch 3:** group on diet Glyferon\* + sucrose solution and paraffin oil; **Batch 4:** group on diet with extract 200 mg/kg + sucrose solution and paraffin oil bw; **Batch 5:** group on diet with extract 400 mg/kg + sucrose solution and paraffin oil bw.

#### **Effect of the extract on body weight gain in rats**

Table 2 shows the influence of the extract on body weight gain in rats. Weight gain was observed in all batches of rats. Over the four (4) weeks, a significant increase in body weight gain was observed in batch 2 (high-calorie diet) compared to group 1 (distilled water). Batch 2 had the highest weight gain, at  $20.9 \pm 4.5$  g. Similarly, a significant increase in body weight gain was observed in batch 2 compared to batches 3, 4, and 5.

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**Table 2:** Influence of the extract on body weight gain in rats.

Body Weight Gain (g)	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
<b>Week 1</b>	2.5 ± 0.3	14.1 ± 4.1 a*k*	2 ± 0.4	0.8 ± 0.1	2.4 ± 0.6
<b>Week 2</b>	3.6 ± 0.4	20.9 ± 4.5 a*k*	3.1 ± 0.2	1.8 ± 0.4	3.2 ± 1.5
<b>Week 3</b>	3.1 ± 0.6	13.1 ± 3.5 a*k*	4.9 ± 1.1	11.5 ± 3.2	6.8 ± 2.2
<b>Week 4</b>	8.1 ± 1.4	12.8 ± 3.4 a*k*	7.9 ± 2.2	7.7 ± 2.1	9.8 ± 3.1

Values are presented as the mean followed by the standard error of the mean (SEM), for each group (n = 6). a: comparison of the control group (group 1) with the high-calorie diet group (group 2); k: comparison of groups 3, 4 and 5 with the high-calorie diet group (group 2); \*p < 0.05: significant difference; **Batch 1:** control with normal diet; **Batch 2:** control with diet with sucrose solution and paraffin oil; **Batch 3:** group with Glyferon diet\* + sucrose solution and paraffin oil; **Batch 4:** group with diet with 200 mg/kg extract + sucrose solution and paraffin oil bw; **Batch 5:** group with diet with 400 mg/kg extract + sucrose solution and paraffin oil bw.

### Effect of the extract on body mass index in rats

Table 3 shows the influence of the extract on the body mass index (BMI) of rats.

Consumption of the high-calorie diet caused a greater increase in body mass index (BMI) values in rats fed this diet alone than in control rats (batch 1) during the experiment. This difference in mean body mass index values was not significantly significant (p > 0.05) from the first to the fourth week of the study.

Rats in the batches that received the high-calorie diet followed by Glyferon and the doses (200, 400 mg/kg body weight) of the extract also experienced increases in body mass index (BMI). However, these increases in the body mass index values of rats in these batches were lower than those of rats in batch 2. The differences between the body mass index (BMI) values of rats that received the doses of the extract (200 and 400 mg/kg body weight) and rats in the other batches (1 and 2) were not statistically significant (p > 0.05).

**Table 3:** Influence of the extract on the body mass index of rats.

Body Mass Index (g/cm <sup>2</sup> )	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
<b>Week 0</b>	3.7 ± 1.4	3.5 ± 1.2	3.6 ± 2.1	3.9 ± 2.1	3.8 ± 3.1
<b>Week 1</b>	4.1 ± 2.3	5.2 ± 2.8	3.9 ± 2.4	4.9 ± 3.1	4.6 ± 1.5
<b>Week 2</b>	4.5 ± 2.2	5.4 ± 3.5	4.1 ± 3.4	5.1 ± 3.2	4.8 ± 3.4

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<b>Week 3</b>	4.6 ± 1.8	5.6 ± 1.6	4.5 ± 2.3	5.3 ± 2.2	5.1 ± 3.7
<b>Week 4</b>	4.7 ± 5.5	5.8 ± 2.6	4.8 ± 3.3	5.5 ± 1.6	5.3 ± 3.5

### Effect of the extract on abdominal circumference in rats

The mean abdominal circumference values of rats fed the high-calorie diet and the aqueous extract of *Lippia multiflora* leaves are shown in Table 4.

Consumption of the high-calorie diet caused a greater increase in abdominal circumference (waist circumference) values in rats (batch 2) fed only this diet than in control rats (batch 1) during the experiment. This difference between the mean waist circumference values was significant from the 2nd to the 4th week.

Rats in the batches that received the high-calorie diet followed by Glyferon and the extract at doses of 200 and 400 mg/kg bw did not experience significant increases in their abdominal circumference values. In addition, these waist circumference values of rats in batches 3, 4 and 5 were significantly lower than those of rats in batch 2 which received only the high-calorie diet.

**Table 4:** Influence of the extract on the abdominal circumference of rats

<b>Waist measurement (cm)</b>	<b>Batch 1</b>	<b>Batch 2</b>	<b>Batch 3</b>	<b>Batch 4</b>	<b>Batch 5</b>
<b>Week 0</b>	11,4 ± 1,2	11,2 ± 2,1	11,9 ± 1,9	11,6 ± 2,2	11,3 ± 2,7
<b>Week 1</b>	11,9 ± 2,1	13,1 ± 2,2	12,4 ± 2,3	12,9 ± 2,3	12,7 ± 1,7
<b>Week 2</b>	12,2 ± 2,2	15,7 ± 3,3 a*k*	12,7 ± 2,7	13,1 ± 3,5	12,9 ± 3,2
<b>Week 3</b>	12,5 ± 3,1	17,4 ± 3,6a*k*	12,9 ± 4,1	13,9 ± 3,6	13,5 ± 3,8
<b>Week 4</b>	12,8 ± 4,1	19,1 ± 2,2a*k*	13,7 ± 4,2	14,9 ± 5,1	14,1 ± 2,4

Values are presented as the mean followed by the standard error of the mean (SEM), for each group (n = 6). a: comparison of the control group (group 1) to the high-calorie diet group (group 2); k: comparison of groups 3, 4 and 4 to the high-calorie diet group (group 2). \*p < 0.05: significant difference; **Batch 1:** control on normal diet; **Batch 2:** control on diet with sucrose solution and paraffin oil; **Batch 3:** group on Glyferon diet\* + sucrose solution and paraffin oil; **Batch 4:** group on diet with 200 mg/kg extract + sucrose solution and paraffin oil bw; **Batch 5**: group on diet with 400 mg/kg extract + sucrose solution and paraffin oil bw

### Effect of the extract on relative mass

Table 5 presents the mean values of the relative weights of the organs (liver, heart, kidneys, visceral adipose tissue, and abdominal subcutaneous adipose tissue).

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In rats fed only the high-calorie diet, the mean value of the relative masses of the liver was higher than that of rats in the control batch ( $8.1 \pm 0.3\%$  vs.  $5.9 \pm 0.4\%$ ). In addition, the relative mass value of rats in the group fed only the high-calorie diet was higher than that of rats treated with the aqueous extract of *Lippia multiflora*.

In terms of relative heart masses, the values were similar from the rats in the untreated high-calorie diet batch ( $0.67 \pm 0.1\%$ ) to the rats in batch 1 ( $0.65 \pm 0.4\%$ ) and to the rats fed a high-calorie diet and treated with the aqueous extract of *Lippia multiflora* leaves at doses of 200 and 400 mg/kg body weight ( $0.68 \pm 0.3$  and  $0.65 \pm 0.4\%$ ).

As for the relative kidney masses, the mean value in rats from batches exclusively subjected to the high-calorie diet was initially higher ( $1.41 \pm 0.1\%$ ) than that of the control batch 1 ( $1.05 \pm 0.2\%$ ) and even higher than the values of the relative kidney masses of rats subjected to the high-calorie diet and treated ( $1.23 \pm 0.6\%$  for 200 mg/kg and  $1.17 \pm 0.4\%$  for 400 mg/kg).

The relative mass of visceral adipose tissue in rats from the control batch 1 ( $0.58 \pm 0.1\%$ ) was lower than that in rats from the untreated high-calorie diet batch ( $1.1 \pm 0.3\%$ ). Rats in batches receiving both the high-calorie diet and the *Lippia multiflora* aqueous extract treatment had lower relative visceral adipose tissue masses ( $0.56 \pm 0.4\%$  for 200 mg/kg and  $0.41 \pm 0.3\%$  for 400 mg/kg) than rats in the high-calorie diet batch.

Abdominal subcutaneous tissues had a significantly higher mean relative mass value ( $8.41 \pm 0.7\%$ ) in rats from the batch that received only the high-calorie diet compared to the mean value of abdominal subcutaneous tissue masses in rats from the control batch 1 ( $4.19 \pm 0.6\%$ ). This difference between the values of relative masses of abdominal subcutaneous adipose tissues was significant ( $p < 0.05$ ). However, the relative mass of abdominal subcutaneous tissues in rats from the batch fed only the high-calorie diet (batch 2) was significantly higher than those in rats from the batches treated with the extract at doses of 200 and 400 mg/kg body weight, respectively ( $4.1 \pm 0.4$  and  $3.87 \pm 0.5\%$ ). The organs of rats treated with the high-calorie diet and Glyferon had relative masses significantly close to those of the organs of rats treated with the high-calorie diet and the extract.

**Table 5:** Influence of the extract on the relative masses of some organs in rats

Values are presented as the mean followed by the standard error of the mean (SEM), for each group (n = 6). a:

Relative masses of organs (%)	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
<b>Liver</b>	$5.9 \pm 0.4$	$8.1 \pm 0.3$	$5.1 \pm 0.1$	$6.1 \pm 1.05$	$5.5 \pm 0.6$
<b>Heart</b>	$0.65 \pm 0.4$	$0.67 \pm 0.1$	$0.64 \pm 0.2$	$0.68 \pm 0.3$	$0.65 \pm 0.4$
<b>Kidneys</b>	$1.05 \pm 0.2$	$1.41 \pm 0.1$	$1.02 \pm 0.4$	$1.23 \pm 0.6$	$1.17 \pm 0.4$
<b>Visceral adipose tissue</b>	$0.58 \pm 0.1$	$1.1 \pm 0.3$	$0.47 \pm 0.2$	$0.56 \pm 0.4$	$0.41 \pm 0.3$
<b>Abdominal subcutaneous adipose tissue</b>	$4.19 \pm 0.6$	$8.41 \pm 0.7$ a*, k*	$3.6 \pm 0.7$	$4.1 \pm 0.4$	$3.87 \pm 0.5$

comparison of the control group (group 1) to the high-calorie diet group (group 2); k: comparison of groups 3, 4 and 4 to the high-calorie diet group (group 2). \* $p < 0.05$ : significant difference; **Batch 1**: control on normal diet; **Batch 2**: control on diet with sucrose solution and paraffin oil; **Batch 3**: group on Glyferon diet\* + sucrose solution

and paraffin oil; **Batch 4:** group on diet with 200 mg/kg extract + sucrose solution and paraffin oil bw; **Batch 5:** group on diet with 400 mg/kg extract + sucrose solution and paraffin oil bw

### 5. DISCUSSION

Our results showed an increase in body weight and weight gain after being fed a high-calorie diet composed of sucrose and paraffin oil. These observations are consistent with studies conducted by (10). According to (13), this increase is due to an accumulation of fats and sugars in the body. Simultaneous treatment of rats with a high-calorie diet and the total aqueous extract of *Lippia multiflora* leaves showed a decrease in body weight and weight gain. These results are consistent with those of (14), who studied the total aqueous extract of *Taraxacum officinale* in rats, and (15), who conducted studies on the aqueous extract of *Tetracera potatoria*.

Our results also showed that the body mass index (BMI) value did not undergo any significant variation in the rats in the batch that was fed only with the high-calorie diet compared to that of the rats in the control batch fed with pellets. In the same way, the body mass index values of the rats in the groups fed with the high-calorie diet and treated with the aqueous extract of *Lippia multiflora* leaves did not undergo any significant variation. These results confirm the observations of the authors who believe that the body mass index (BMI) is not sufficient on its own to diagnose obesity but that it must be associated with other markers such as waist circumference and lipid profile (16). The plant extract reduced abdominal circumference (waist circumference), which appeared to increase with the high-calorie diet. These results are consistent with those of (17), who conducted a double-blind study in 50 obese men, with a reduction in waist and hip circumference and induced weight loss after administration of *Nigella sativa* extract.

The study of the effect of *Lippia multiflora* aqueous extract on the relative mass of the heart, kidneys, liver, and visceral adipose tissue showed non-significant variations between the batches of rats studied. However, a significant difference in relative mass due to an increase in the relative mass of abdominal subcutaneous adipose tissue in rats fed exclusively on the high-calorie diet should be noted. This increase in the relative mass of abdominal subcutaneous adipose tissue may be the result of an accumulation of fat deposited in the abdominal muscle. This explains the high average waist circumference value in animals that consumed the high-calorie diet without the addition of aqueous extract of *Lippia multiflora* leaves. It has been shown by the studies of (18) that excessive intake of lipids through diet can promote their accumulation in striated muscles and adipose tissues. On the other hand, a significant decrease in the relative mass of abdominal subcutaneous adipose tissue is noted in rats receiving both the high-fat diet and the aqueous extract of *Lippia multiflora*, compared to rats fed only the high-calorie diet. The aqueous extract of *Lippia multiflora* could therefore prevent the deposition of fats in striated muscles and adipose tissues.

### 6. CONCLUSION

The high-calorie diet induced obesity in rats over a period of twenty-eight (28) days. This diet caused an increase in body weight. This observation validates the hypothesis of an obesogenic effect of this diet. This diet, characterized by excessive calorie intake, also caused disturbances in anthropometric parameters and the relative masses of certain organs such as the liver, heart, kidneys, and adipose tissue.

The results of the experiment conducted on rats subjected to the same high-calorie diet demonstrated a beneficial effect of the aqueous extract of *Lippia multiflora* leaves by correcting anthropometric parameters and relative organ masses.

Our results therefore confirm the anti-obesity effect of the aqueous extract of *Lippia multiflora* leaves. This effect could be due to the chemical compounds contained in *Lippia multiflora* leaves.

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