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Evaluation of the preventive effect of the total aqueous extract of *Lippia multiflora* Moldenke leaves on organs 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 certain organs in rats made obese by a hypercaloric diet.

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. The organs are then isolated to assess their weight and physical condition.

Results: The extract 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. the organs also gradually regained their physical condition.

Conclusion: In summary, *Lippia multiflora* possesses anti-obesity effects, because it preserves organs after obesity induction.

Keywords: *Lippia multiflora*; Obesity; Rats; Organs; Relative Masses

1. Introduction

Obesity is now considered a pandemic. Indeed, the WHO has declared it a global public health problem because the obesity epidemic continues unabated in Western countries and is increasing more dramatically in the rest of the world, paradoxically even in countries where poverty and malnutrition are widespread. Obesity rates are dramatically increasing, not only in adult populations, but also among children. Indeed, the increase in average weight is observed across all age groups and socioeconomic groups [1].

Obesity is a multifactorial disease involving genetic, behavioral, and endocrine factors [2]. It is a complex chronic condition characterized by excess fat deposits that can be detrimental to health. Obesity can increase the risk of type 2 diabetes and heart disease, can affect bone health and reproductive performance, and increases the risk of certain cancers. Obesity affects quality of life, such as sleep and mobility [3].

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Obesity is a global epidemic that causes 25 million deaths worldwide each year [4]. It is a serious condition that leads to numerous health problems, such as musculoskeletal disorders, cardiovascular diseases, type 2 diabetes, and various types of cancer [5]. It also has a significant impact on an individual's social, financial, and psychological status, which can contribute to the development of depression [6]. According to [7], it is characterized by significant excess weight due to an excessive amount of lipids stored in white adipose tissue, and it represents a determining risk factor in the development of non-insulin-dependent diabetes and cardiovascular diseases.

Obesity has several targets in the body, particularly on certain organs. According to [8], the kidneys, heart, liver and adipose tissue are affected in an obese subject.

Faced with this spectacular advance in obesity and the impotence of modern medicine, plants have become a safer alternative for populations. Several plants are used in the traditional treatment of obesity. It is important to provide a scientific basis for these traditional uses. It is in this context that we investigated the potential anti-obesity effect of the aqueous extract of *Lippia multiflora* leaves on organs such as the liver, kidneys, heart, and adipose tissue in rats made obese by a high-fat diet.

2. Material

2.1. 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.

2.2. 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

3.1. 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].

3.2. 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 reported by [10,11]. 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.

3.3. Sampling and macroscopic examination of some organs

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].

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

3.4. Statistical Analysis

Data were expressed as the mean followed by the standard error of the mean (M ± 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 Prsim 8.0 software (San Diego, California, USA) was used for statistical analysis.

4. Results

4.1. 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%.

4.2. Effect of the extract on relative mass

Table 1 presents the mean values of the relative weights of the organs (liver, heart, kidneys, visceral adipose tissue, and abdominal subcutaneous adipose tissue).

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 ± 0.3% vs. 5.9 ± 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 ± 0.1%) to the rats in batch 1 (0.65 ± 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 ± 0.3 and 0.65 ± 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 ± 0.1%) than that of the control batch 1 (1.05 ± 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 ± 0.6% for 200 mg/kg and 1.17 ± 0.4% for 400 mg/kg).

The relative mass of visceral adipose tissue in rats from the control batch 1 (0.58 ± 0.1%) was lower than that in rats from the untreated high-calorie diet batch (1.1 ± 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 ± 0.4% for 200 mg/kg and 0.41 ± 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 ± 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 ± 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 ± 0.4 and 3.87 ± 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.

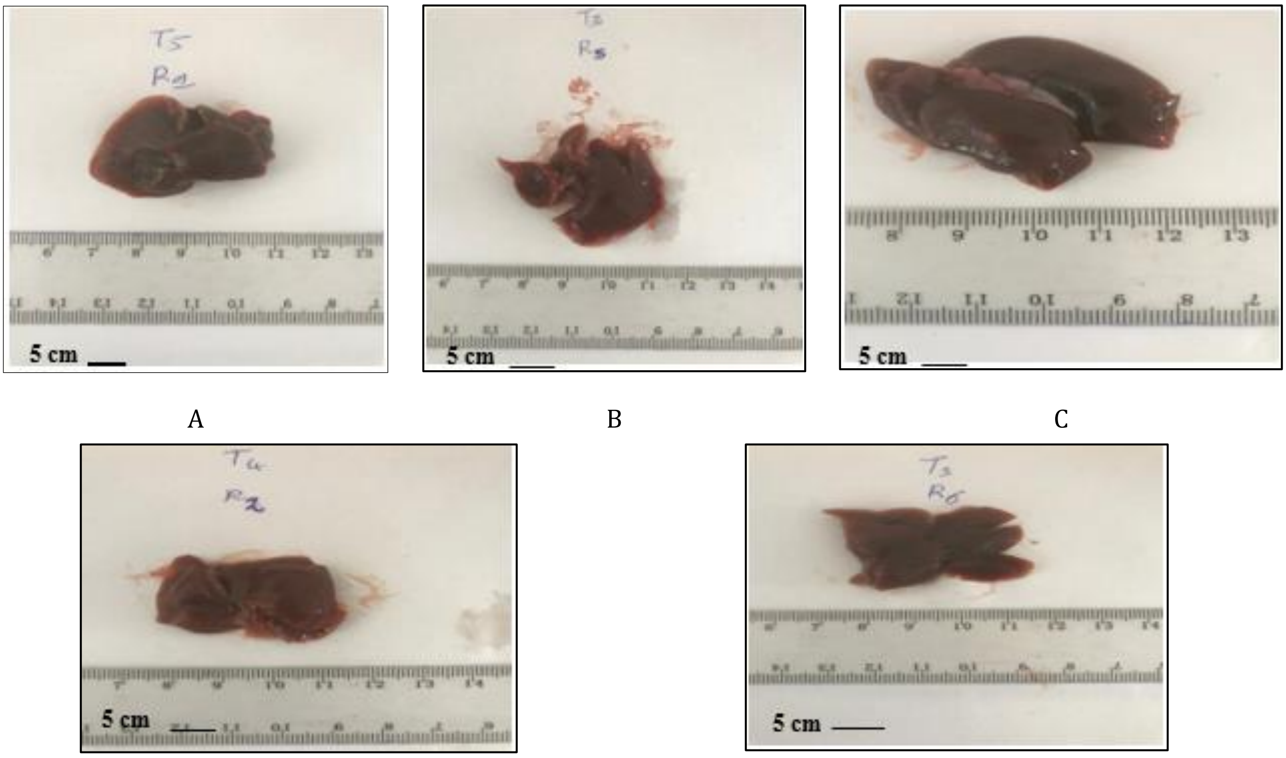
Table 1 Influence of the extract on the relative masses of some organs in rats

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

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

4.3. Effect of the extract on macroscopic histological observations of some organs of rats

Macroscopic observation of the rats tested revealed steatosis in the livers of rats fed only the high-calorie diet. In addition, fat was found in the livers of rats fed the high-calorie diet without treatment with the aqueous extract of *Lippia multiflora* leaves. These obesity markers were not observed in the livers of rats in the control batch (1) or in the livers of rats in the batches that received the high-calorie diet and treated with different doses of the aqueous extract of *Lippia multiflora* leaves (figure 1).



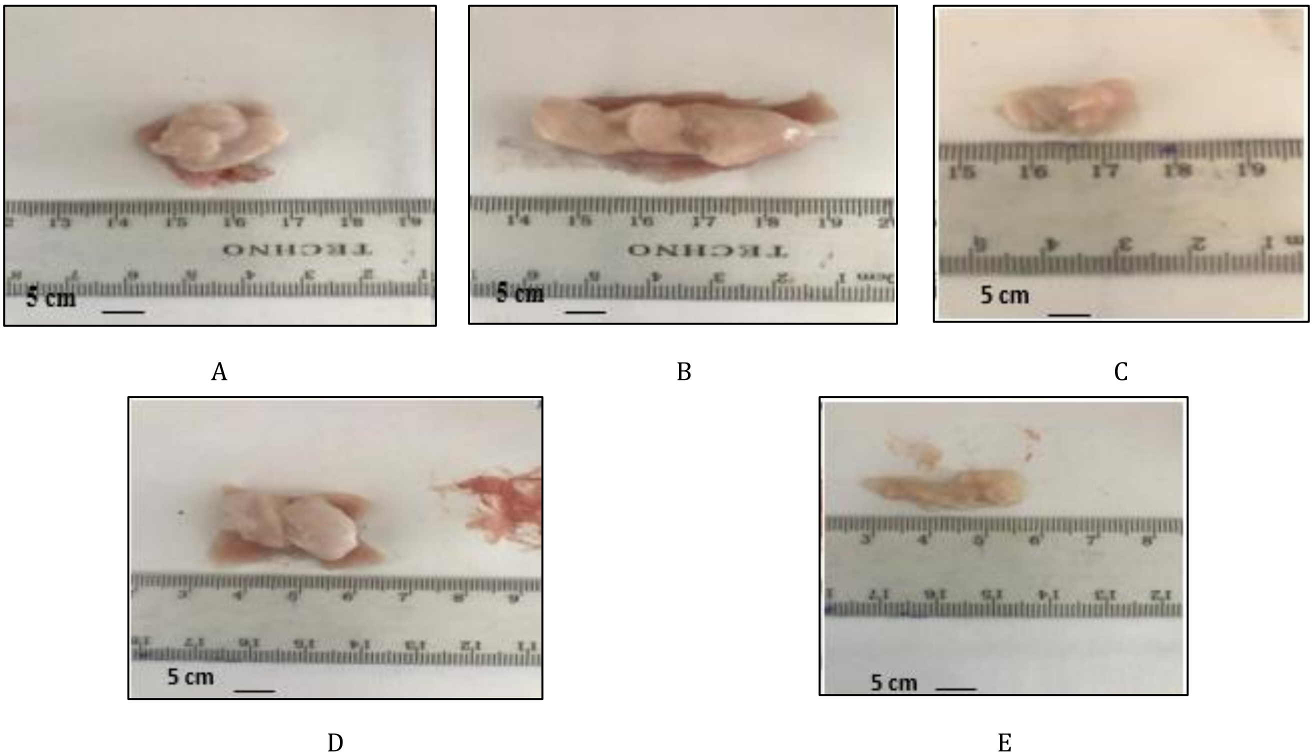
A B C

D E

A: batch (1) control; B: batch (2) high-calorie diet only; C: batch 3 (high-calorie diet and Glyféron at 0,1 mg/kg); D: batch 4 (high-calorie diet and extract at 200 mg/kg) and E: batch 5 (high-calorie diet and extract at 400 mg/kg). The images are representative of the livers of the different groups of rats after 28 days of study.

Figure 1 Photograph of the livers of *Wistar* rats fed a high-calorie diet and treated for 28 days

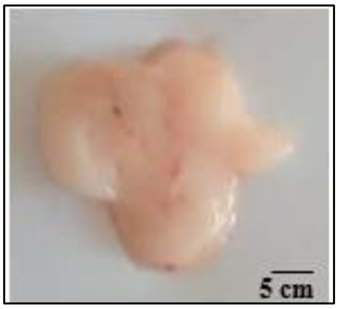
Macroscopic histological examination of adipose tissue revealed an increase in abdominal fat mass in rats fed exclusively a high-calorie diet, compared to adipose tissue collected from the abdominal area of control rats. In contrast, administration of a high-calorie diet with aqueous extract of *Lippia multiflora* at different concentrations (200 mg/kg and 400 mg/kg) resulted in a normal reorganization of adipose tissue architecture, as illustrated in the attached photographs (figure 2).

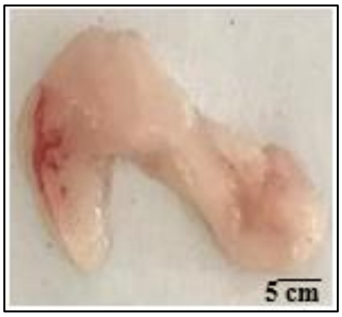


A: batch 1 (control); B: batch 2 (high-calorie diet only); C: batch 3 (high-calorie diet and Glyféron at 0,1 mg/kg); D: batch 4 (high-calorie diet and extract at 200 mg/kg) and E: batch 5 (high-calorie diet and extract at 400 mg/kg).

Figure 2 Photograph of adipose tissues of *Wistar* rats fed a high-calorie diet and treated for 28 days

Macroscopic histological examination of adipose tissue revealed an increase in visceral fat mass in rats fed exclusively a high-calorie diet, compared to adipose tissue collected from the visceral area of control rats. In contrast, administration of high-calorie diet plus aqueous extract of *Lippia multiflora* at different concentrations (200 mg/kg and 400 mg/kg) resulted in normal reorganization of adipose tissue architecture, as illustrated in the attached photographs (figure 3).





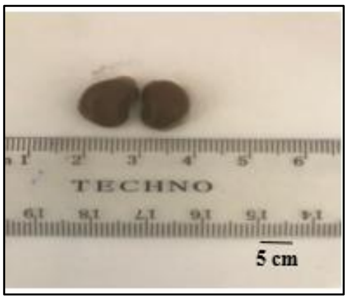
A B C

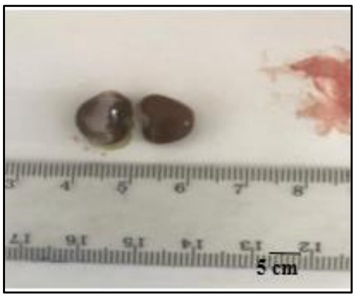
D E

A: batch 1 (control); B: batch 2 (high-calorie diet only); C: batch 3 (high-calorie diet and Glyféron at 0,1 mg/kg); D: batch 4 (high-calorie diet and extract at 200 mg/kg) and E: batch 5 (high-calorie diet and extract at 400 mg/kg).

Figure 3 Photograph of visceral adipose tissues of *Wistar* rats fed a high-calorie diet and treated for 28 days

Microscopic histological analysis of the kidneys of the tested rats revealed an accumulation of fat in the kidneys of rats fed exclusively on a high-calorie diet, compared to the kidneys of control rats and rats fed on a high-calorie diet, but supplemented with the aqueous extract of *Lippia multiflora* at various concentrations. This study highlights a structural reorganization of adipose tissues, as illustrated in the attached images (figure 4).





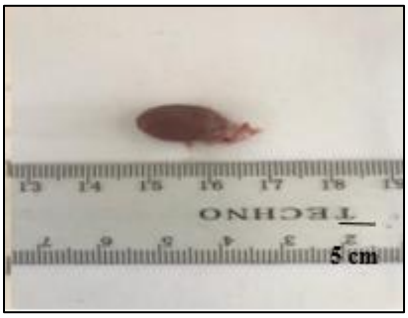
A B C

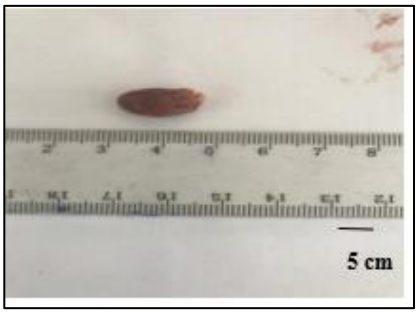
D E

A: batch 1 (control); B: batch 2 (high-calorie diet only); C: batch 3 (high-calorie diet and Glyféron at 0,1 mg/kg); D: batch 4 (high-calorie diet and extract at 200 mg/kg) and E: batch 5 (high-calorie diet and extract at 400 mg/kg).

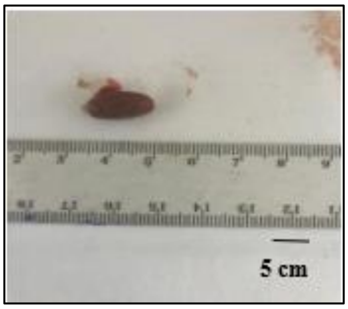
Figure 4 Photograph of kidneys of *Wistar* rats fed a high-calorie diet and treated for 28 days

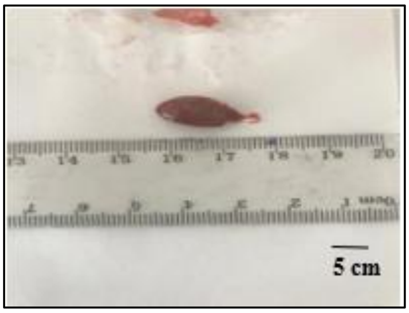
Microscopic histological examination of the hearts of rats fed specific diets revealed an accumulation of fat in the hearts of animals fed exclusively on a high-calorie diet, compared with the hearts of control specimens and rodents fed the same diet, but supplemented with various concentrations of aqueous extract of *Lippia multiflora* (figure 5).





A B C





D E

A: batch 1 (control); B: batch 2 (high-calorie diet only); C: batch 3 (high-calorie diet and Glyféron at 0,1 mg/kg); D: batch 4 (high-calorie diet and extract at 200 mg/kg) and E: batch 5 (high-calorie diet and extract at 400 mg/kg).

Figure 5 Photograph of hearts of *Wistar* rats fed a high-calorie diet and treated for 28 days

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.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

There is no conflict of interest for these studies.

Statement of ethical approval

The equipment usage handling and sacrificing of the animals were performed in accordance with the European Council legislation 87/609/EEC for the protection of experimental animals

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Authors' contributions

The corresponding author participated in funding and handling. The other two authors participated in funding and processing of results.

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