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EVALUATION OF THE PREVENTIVE EFFECT OF THE TOTAL AQUEOUS EXTRACT OF LIPPIA MULTIFLORA LEAVES ON BIOCHEMICAL PARAMETERS IN RATS MADE OBESE BY A HIGH-CALORIE DIET

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ABSTRACT
Obesity has become a serious public health problem, with modern treatment being lengthy and costly, often inaccessible to all patients. Traditional medicine therefore offers an important alternative for low-income populations. The objective of this study was to evaluate the effect of Lippia multiflora aqueous extract on obesity prevention through biochemical parameters indicative of obesity. 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 extract at these different doses showed a decrease in biochemical parameters such as triglycerides, total cholesterol, and LDL cholesterol, associated with an increase in HDL cholesterol. These parameters had opposite kinetics after administration of the high-calorie diet. These observed changes were significant at doses of 200 and 400 of the extract. Ultimately, Lippia multiflora possesses anti-obesity effects, allowing it to prevent the onset of obesity due to a high-calorie diet by stabilizing biochemical parameters.

KEYWORDS: Lippia multiflora, Obesity, rats, biochemical parameters.

INTRODUCTION
Obesity is a public health problem that is becoming a pandemic worldwide. It is defined as a metabolic disorder characterized by excessive fat accumulation in the body due to energy intake exceeding energy expenditure.[1] This accumulation of fat in tissues increases the risk of numerous pathologies such as diabetes, hypercholesterolemia, and hypertension.[2] A diagnosis of a body mass index greater than 30 kg/m2 in men indicates obesity. Worldwide, since the 1980s, the prevalence of obesity has more than doubled, and today, nearly one in ten adults is obese.[3] The number of adults in 2008 who were overweight was 1.46 billion, with 205 million men and 297 million women obese.[4] The African continent is also affected by this pandemic, but especially among children. According to,[5] in 2019 the continent was home to 24% of children under 5 years of age who were overweight in the world. Studies carried out by.[6] in the school environment in Côte d'Ivoire showed a prevalence of 5%. They observed 4% of subjects who were overweight, 39% who were underweight, 25% who were thin, and 27% who were normal. The first recommendations to prevent or manage obesity are firstly a modification of nutritional habits (low fat, sugar and sodium content), regular exercise and finally, drug treatment with conventional products. However, drug treatment has shown that safety, inefficacy, tolerability and significant adverse effects of currently available anti-obesity drugs occur during long-term use.[7,8] It is in this context that plants have become an interesting alternative for our populations to alleviate several pathologies such as obesity. It is in this perspective that we undertook to study the effect of the aqueous extract of the leaves of Lippia multiflora (Verbenaceae), a plant of the Ivorian pharmacopoeia, on the prevention of obesity. Concretely, it is a question of studying the potential effect of this extract on clinical biochemical markers, in rats made obese by a hypercaloric diet.

MATERIALS
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.

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 the Aqueous Extract of Lippi Multiflora Leaves on Obesity Induced by a High-Calorie Diet in Rats

We implemented this method based on the studies of.[10] The aim 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) groups of six (6) rats each and then treated them by gavage (oral route) with different products:

Group 1: Rats given distilled water at a dose of 5 mL/kg bw;

Group 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);

Group 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);

Batch 4 and 5 (batches tested): Rats having received respectively the aqueous extract at doses of 200 and 400 mg/Kg of bw + the solution (sucrose at 30% at 10 mL/Kg, bw + refined palm oil at 10 mL/Kg, bw).

Blood and Organ Collection

Blood samples were collected from all rats at the beginning (D0) and at the end of the experiment (28 days). After a 12-hour fast, venous blood was collected from the retroorbital sinus of each animal's eye using a sterile Pasteur pipette. A blood sample (approximately 2 mL) was then collected and immediately placed in dry tubes for biochemical analyses.[11] LDL cholesterol levels were calculated using the following formula used by.[10]

LDL = total cholesterol − HDL cholesterol − triglyceride / 5.

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.

RESULTS

Effect of the extract on biochemical parameters

Effect of the extract on Triglycerides

A significant increase in the concentration of triglycerides was observed in the group fed only with the high-calorie diet (from 1.5 ± 0.2 to 1.9 ± 0.1 g/L) compared to the concentration of triglycerides in the rats in the control group (from 1.6 ± 0.4 to 1.65 ± 0.3 g/L).

As for the triglyceride concentrations of rats receiving the high-calorie diet and the aqueous extract of Lippia multiflora at different doses (200 and 400 mg/kg), they underwent slight increases (from 1.5 ± 0.1 to 1.62 ± 0.2 and 1.61 ± 0.5 to 1.68 ± 0.3 g/L) compared to those of rats in the group that received only the high-calorie diet. The same kinetics of action were observed in group 2 (see Figure 1).

Effect of the extract on total cholesterol

The total cholesterol concentration of rats fed only the high-calorie diet (group 2) (0.47 ± 0.1 to 1.41 ± 0.2 g/L) increased more than the total cholesterol concentration in rats in group 1 (0.48 ± 0.1 to 0.56 ± 0.1 g/L) that were not fed the high-calorie diet. This difference in the increases in mean values of total cholesterol concentrations was significant.

Serum total cholesterol concentrations in rats receiving both the high-fat diet and the aqueous extract of Lippia multiflora at different doses (200 and 400 mg/kg) showed significantly significant decreases (from 0.46 ± 0.4 to 0.74 ± 0.3 g/L and from 0.47 ± 0.2 to 0.61 ± 0.2 g/L). The same kinetics of action were observed in batch 2 (Figure 2).

Effect of the extract on HDL cholesterol

The HDL cholesterol concentration of rats in group 2 fed the high-calorie diet (from 0.16 ± 0.06 to 0.10 ± 0.04 g/L) decreased significantly more than that of rats in the control group (from 0.15 ± 0.02 to 0.14 ± 0.17 g/L) between the day before the experiment and the day the experiment ended. Furthermore, HDL-cholesterol concentrations in rats receiving the high-calorie diet and treated with the aqueous extract of Lippia multiflora at different doses (200 and 400 mg/kg) showed significantly increased (from 0.15 ± 0.04 to 0.19 ± 0.02 and 0.16 ± 0.04 to 0.22 ± 0.02) between the beginning and the end of the experiment. The same kinetics of action were observed in batch 2 (Figure 3).

Effect of the extract on LDL-cholesterol

The LDL-C concentration in group 2 fed the high-fat diet increased significantly (0.25 ± 0.5 to 0.98 ± 0.4 g/L), while the serum LDL-cholesterol concentration decreased in group 1 (0.24 ± 0.3 to 0.26 ± 0.1 g/L) between before and after the experiment. As for the LDL-cholesterol concentrations in the blood of rats receiving the high-fat diet and the aqueous extract of Lippia multiflora at different doses (200 and 400 mg/kg), they obtained respective significant decreases (0.25 ± 0.3 to 0.21 ± 0.4 g/L and 0.25 ± 0.2 to 0.21 ± 0.3 g/L) in contrast to the LDL-cholesterol concentration values of rats in batch 2 (high-fat diet only). The same kinetics of action are observed in batch 2 (Figure 4).

Effect of the extract on blood glucose

The blood glucose levels of rats in the group fed only the high-calorie diet increased significantly (from 0.58 ± 0.06 to 1.34 ± 0.04 g/L) compared to the blood glucose levels of rats in the control group (0.55 ± 0.06 to 0.57 ± 0.04 g/L). As for the blood glucose values of rats receiving the high-calorie diet and the aqueous extract of Lippia multiflora at different doses (200 and 400 mg/kg), they had significant increases (0.51 ± 0.06 to 0.81 ± 0.07 g/L and 0.52 ± 0.06 to 0.78 ± 0.04 g/L) as well as those of rats in batch 2. The same kinetics of action were observed in batch 2 (figure 5).

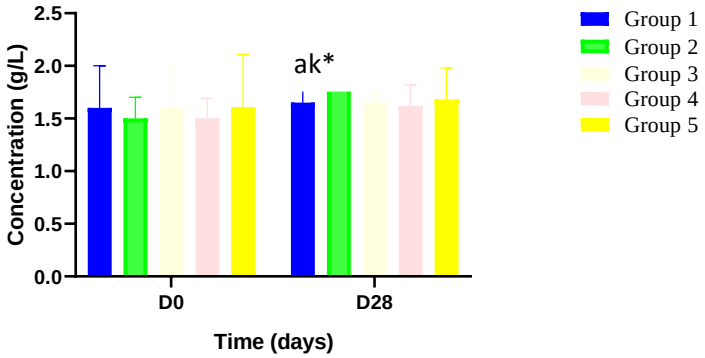

Figure 1: Influence of the extract on triglyceride concentrations in rats.

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; Group 1: control on the normal diet; Group 2: control on the diet with sucrose solution and paraffin oil; Group 3: group on the Glyferon diet* + sucrose solution and paraffin oil; Group 4: group on the diet with the 200 mg/kg extract + sucrose solution and paraffin oil bw; Batch 5: batch on diet with extract 400 mg/kg + sucrose solution and paraffin oil b.w., D: day.

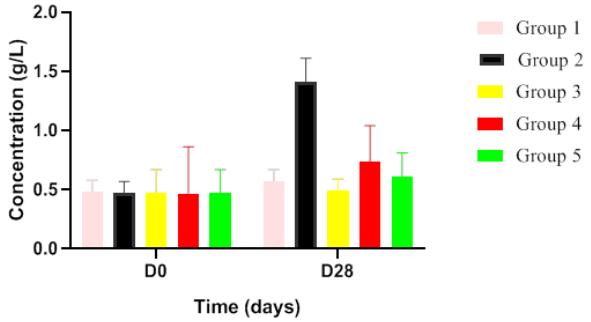

Figure 2: Influence of the extract on total cholesterol concentrations in rats.

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 fatty diet group (group 2); k: comparison of groups 3, 4, and 4 to the fatty diet group (group 2); m: comparison between groups 3, 4, and 5; *p < 0.05, **p < 0.01: significant difference; Group 1: control on the normal diet; Group 2: control on the diet with sucrose solution and paraffin oil; Group 3: group on the Glyferon diet* + sucrose solution and paraffin oil; Group 4: group on the diet with the 200 mg/kg extract + sucrose solution and paraffin oil bw; Batch 5: batch on diet with extract 400 mg/kg + sucrose solution and paraffin oil b.w., D: day.

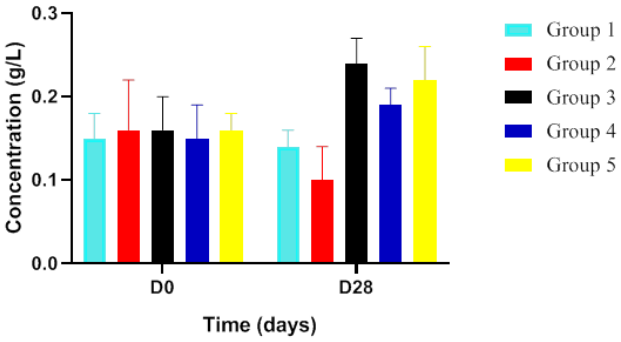

Figure 3: Influence of the extract on HDL-c cholesterol concentrations in rats.

HDL-c: HDL cholesterol; 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 fatty diet group (group 2); k: comparison of groups 3, 4 and 4 to the fatty diet group (group 2); *p < 0.05: significant difference; Group 1: control on normal diet; Group 2: control on diet with sucrose solution and paraffin oil; Group 3: group on Glyferon diet* + sucrose solution and paraffin oil; Group 4: group on diet with extract 200 mg/kg + sucrose solution and paraffin oil bw; Batch 5: batch on diet with extract 400 mg/kg + sucrose solution and paraffin oil b.w., D: day

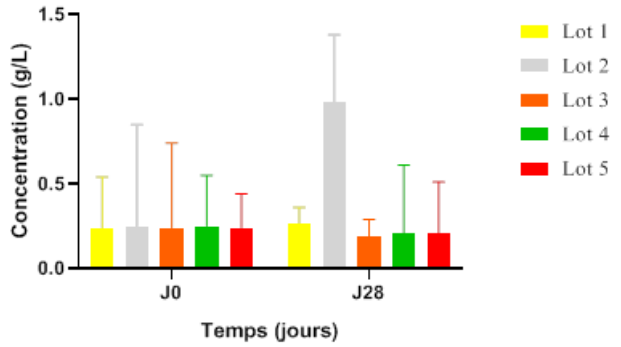

Figure 4: Influence of the extract on LDL-C cholesterol concentrations in rats.

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 fatty diet group (group 2); k: comparison of groups 3, 4, and 4 to the fatty diet group (group 2); *p < 0.05; **p < 0.01: significant difference; Group 1: control on the normal diet; Group 2: control on the diet with sucrose solution and paraffin oil; Group 3: group on the Glyferon diet* + sucrose solution and paraffin oil; Group 4: group on the diet with the 200 mg/kg extract + sucrose solution and paraffin oil bw; Batch 5: batch on diet with extract 400 mg/kg + sucrose solution and paraffin oil b.w., D: day.

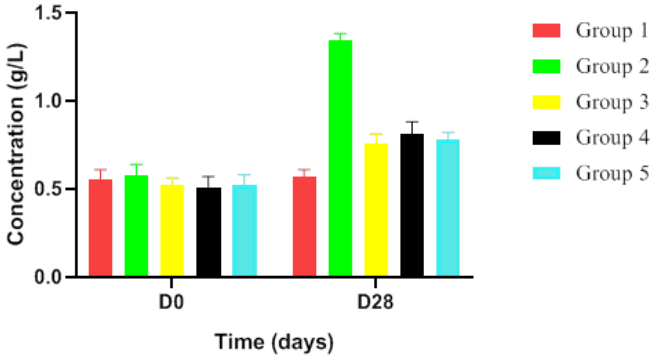

Figure 5: Influence of the extract on blood glucose levels in rats.

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 fatty diet group (group 2); k: comparison of groups 3, 4, and 4 to the fatty diet group (group 2); m: comparison between groups 3, 4, and 5; *p < 0.05; **p < 0.01: significant difference Group 1: control on the normal diet; Group 2: control on the diet with sucrose solution and paraffin oil; Group 3: group on the Glyferon diet* + sucrose solution and paraffin oil; Group 4: group on the diet with the 200 mg/kg extract + sucrose solution and paraffin oil bw; Batch 5: batch on diet with extract 400 mg/kg + sucrose solution and paraffin oil b.w., D: day.

DISCUSSION

Our results showed significant increases in serum concentrations of triglycerides, total cholesterol, LDL cholesterol, and blood glucose, and a significant decrease in HDL cholesterol concentrations in rats fed only the high-calorie diet. These results are consistent with the work of,[10] who observed significant changes in these parameters after rats were placed on a high-calorie diet. Also, the findings of[12] after studies in a group of adolescents recruited with high BMIs confirmed a state of obesity by discovering elevated concentrations of total cholesterol, LDL cholesterol, and triglycerides in the blood serum samples. According to,[13] an increase in the levels of total cholesterol, triglycerides, LDL-cholesterol and a concomitant decrease in HDL-cholesterol are characteristic of dyslipidemia. On the other hand, serum lipid parameters and blood glucose had opposite kinetics in rats that were fed simultaneously with the high-fat diet and Lippia multiflora extract. The aqueous extract of Lippia multiflora leaves at these different doses therefore significantly decreased the serum concentrations of triglyceride, total cholesterol, LDL-cholesterol, glucose and increased the concentrations of HDL-cholesterol in rats fed with the high-calorie diet and treated with the extract during the 28 days of the experiment. These results are in agreement with those of[14] with the aqueous extract of Nigella sativa in Psammomys obesus, of[15] with carob powder and[10] with the aqueous extract of Tetracera potatoria leaves in rats.[16] studies showed the presence of saponosides, tannins, flavonoids, alkaloids and phenols in the leaves of Lippia multiflora. Indeed, the flavonoids present in the aqueous extract of Lippia multiflora according to[17] may be responsible for the antioxidant effects observed in rats subjected to a high-calorie diet. Indeed, flavonoids inhibit the presence of free radicals and oppose the oxidation of macromolecules such as proteins.[18] Saponosides, tannins, and flavonoids are important anti-obesity substances due to their role as dietary antioxidants in preventing oxidative damage in living systems[19] mainly in the regulation of adipose tissue. They also inhibit the growth of 3T3-L1 preadipocytes in vitro, induce adipocyte apoptosis, and inhibit lipid accumulation[20,21] and inhibit the uptake of pancreatic lipase and fatty acids in vivo.

CONCLUSION

The high-calorie diet induced obesity in rats over a period of twenty-eight (28) days. This observation validates the hypothesis of an obesogenic effect of this diet. This diet caused lipid metabolism disturbances through hypercholesterolemia and hyperglycemia in rats.

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 the concentrations of total cholesterol, glucose, triglycerides, LDL-C, and HDL-C.

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.

Competing interests : The authors declare that there are no competing interests.

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