Scholars Academic Journal of Pharmacy
Abbreviated Key Title: Sch Acad J Pharm
ISSN 2347-9531 (Print) | ISSN 2320-4206 (Online)
Journal homepage: http://saspublishers.com

OPEN ACCESS

Biological Sciences

Therapeutic Potential of *Citrus limon* (Rutaceae) on Induced Obesity in Wistar Rats

EKISSI Yapi Hugues Romaric[1*], N'DOUA Akouah Richmonde Leatitia[2], COULIBALY Djeneba[3], ABO Kouakou Jean-Claude[3]

[1]Laboratory of Biological Sciences Animals; specialty Animal Physiology, Phytotherapy and Pharmacology; Science and Technology Training and Research Unit; Alassane OUATTARA University, Bouaké, Ivory Coast
[2]Laboratory of Biodiversity and Tropical Ecology, Speciality Physiology Animal and Pharmacology, University Jean Lorougnon Guédé, Daloa, Ivory Cost
[3]Laboratory of Biology and Health; Animal Physiology, Phytotherapy and Pharmacology Specialty; Felix Houphouët Boigny University, Abidjan, Ivory Coast

DOI: https://doi.org/10.36347/sajp.2025.v14i09.005 | **Received:** 04.10.2025 | **Accepted:** 26.11.2025 | **Published:** 29.11.2025

***Corresponding author:** EKISSI Yapi Hugues Romaric
Laboratory of Biological Sciences Animals; specialty Animal Physiology, Phytotherapy and Pharmacology; Science and Technology Training and Research Unit; Alassane OUATTARA University, Bouaké, Ivory Coas

Abstract **Original Research Article**

To contribute to the valorization of plants used in traditional medicine for improving population health, this study was conducted on *Citrus limon* (Rutaceae), a plant used in Côte d'Ivoire to treat various conditions, including obesity. The acute toxicity study of the aqueous extract of *Citrus limon* (EACl), performed by gavage in female mice according to OECD 423 guidelines, shows that administration of EACl, up to the maximum dose of 5000 mg/kg body weight, does not result in any mortality of the treated animals. This leads to the conclusion that EACl is not toxic orally. A study of the effects of EACl on obesity induced in rats by a high-calorie, high-fat diet shows that this extract leads to a decrease in body weight in obese rats and has anti-hyperlipidemic and anti-hypercholesterolemic effects in these obese animals treated with the extract at a dose of 1000 mg/kg BW. These effects of EACl on obesity are similar to those of atorvastatin (10 mg/kg BW), a reference anti-hypercholesterolemic substance. EACl could therefore act against obesity through a mechanism similar to that of this statin. These effects of EACl justify the use of *Citrus limon* (Rutaceae) in traditional medicine for the treatment of obesity.

Keywords: *Citrus limon*, obesity, anti-hyperlipid, anti-hypercholesterolemic.

INTRODUCTION

Obesity is an excess of body fat, which has consequences for health and reduces life expectancy. It constitutes one of the most serious public health problems in the world (Leyvraz *et al.*, 2008).

In Côte d'Ivoire, 32.2% of the total population is overweight, of which 9.1% are obese. This is largely due to the Westernization of the diet. Indeed, experimental and epidemiological data suggest that a diet high in fat promotes the development of obesity and that there is a direct correlation between fat intake and the degree of obesity (Ailhaud, 2008).

Medicinal plants have always been used to prevent or treat various diseases. They contain bioactive molecules with multiple applications in different fields. Among these compounds, secondary metabolites are particularly useful in therapeutics (Anderson and Markham, 2006). *Citrus limon* (Rutaceae), a medicinal plant used in traditional medicine to treat various ailments, is known to be effective in treating obesity.

Therefore, this study aims to evaluate the therapeutic potential of *Citrus limon* for induced obesity in rats.

I- MATERIALS AND METHODS

1. MATERIALS

1.1- Plant material

The plant material consists of leaves of *Citrus limon* (Rutaceae) purchased at the Cocody market (Abidjan, Côte d'Ivoire). This plant was identified and authenticated at the National Floristic Center (CNF) of the Félix HOUPHOUËT-BOIGNY University.

1.2 - Animal Material

Mus musculus mice (Muridae), of homogeneous Swiss parental strains, weighing between 20 g and 25 g, are used for toxicity testing. These animals are kept at the animal facility of the UFR Biosciences at Félix HOUPHOUËT-BOIGNY University, at room temperature and under daylight and darkness. They are fed pellets supplied by Ivograin and have free access to water.

Wistar rats *Rattus norvegicus* (Muridae), weighing between 180 g and 200 g, reared under the same conditions as mice, are used for pharmacological studies on obesity in rats.

All experimental protocols on these animals are conducted in accordance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes and Commission Recommendation 2007/526/EC concerning guidelines for the housing and care of animals used for experimental or other scientific purposes (Anonymous, 2010).

1.3 - Pharmacological Substances and Physiological Solutions

For our work, we used a reference anti-hypercholesterolemic drug, Atorvastatin (Fluka, Germany), as the pharmacological substance.

2 - METHODS

2.1 - Method for Preparing the Aqueous Extract of *Citrus limon* (Rutaceae) Leaves

To prepare the aqueous extract of *Citrus limon*, 100 g of fresh leaves of this plant are boiled for 15 minutes in 1 L of distilled water. The resulting decoction is filtered twice through absorbent cotton, then twice through Whatman No. 2 filter paper. The collected filtrate is dried in an oven (Thermo SCIENTIFIC VT 6060 M) at 40 °C for 72 hours. After drying, the aqueous extract of *Citrus limon* leaves (EACl) is in powder form. This product (EACl) is used for toxicity testing in mice and pharmacological studies on obesity in rats.

2.2 - Study of the acute toxicity of the aqueous extract of *Citrus limon* (Rutaceae)

The oral toxicity study of the aqueous extract of *Citrus limon* (EACl) is carried out according to OECD guidelines 423 (OECD, 2001).

For the study, three groups of three non-pregnant female mice were placed in cages. The average weight of each group was determined. The tests involved one control group and two test groups. Each mouse received, via gavage using a gastric tube connected to a syringe, 1 ml of a single dose of EACl, expressed in mg/kg body weight (mg/kg BW), with predefined doses of 2000 and 5000 mg/kg body weight (BW) were administered to the two test groups. The different doses of the extract under study were prepared with distilled water. The mice in the control group each received 1 ml of distilled water.

The experiment was conducted sequentially, group by group. First, the mice in the first test group received a single dose of 2000 mg/kg BW. They were then observed for 24 hours, with particular attention paid during the first 4 hours following gavage. The effects of the extract on the behavior of the treated animals were observed, and any symptomatic toxicity was noted. The number of deaths was also recorded. Observation of the animals and the counting of deaths continued for 14 days.

The absence of mortality observed in the animals of this first group led to the administration of the higher dose, which is the limit dose of 5000 mg/kg body weight, to the second group. The mice in this group were also observed, and the number of deaths was also recorded.

2.3 - Pharmacological methods for studying obesity in rats

2.3.1 - Induction of obesity in rats

After an adaptation period (28 days), obesity was induced in male rats using a high-fat, high-calorie diet (Louala, 2017). After consuming the obesogenic (high-calorie, high-fat) diet, rats whose weight had increased by 50% were considered obese and included in the obese rat studies.

2.3.2 - Studies of the Effects of EACl and Atorvastatin on Obese Rats

In this study, six (6) groups of ten (10) rats were formed, consisting of one group of healthy rats and five (5) groups of obese rats:

These groups are treated as follows:

- Group I: The rats in this group are healthy and receive distilled water, then a standard diet. This group serves as the healthy control (St-H_2O + R-St).
- Group II: The rats in this group are obese and receive a standard diet. This group serves as the obese (untreated obese) control (Ob-H_2O + R-St).
- Group III: The rats in this group are obese and receive the high-fat, high-calorie diet. This group of obese rats continues to receive the obesogenic diet (Ob-H_2O + R-Hyp).
- Group IV: The rats in this group are obese and treated with atorvastatin 10 mg/kg body weight, then receive the high-fat, high-calorie diet (Ob-At 10 + R-Hyp).
- Groups V and VI: the rats in these groups are obese and treated respectively with EACl at 750 and 1000 mg/kg of body weight, then receive a hyperlipidemic and hypercaloric diet (Ob-EACl 750 + R-Hyp and Ob- EACl 1000 + R-Hyp).

Body composition (mass) and blood parameters (total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides) are determined before treatment (Day 0), then every 7 days until Day 28 of the experiment.

2.3-3- Determination of Biochemical Parameters

2.3-3-1- Triglyceride Assay

Triglyceride assay is an enzymatic and colorimetric assay performed using the method of Fossati and Prencipe (1982) (Biomaghreb Kit, Tunis, Ref. 20131). The intensity of the pink color is proportional to the triglyceride concentration. This color is measured using a spectrophotometer at a wavelength of 505 nm.

2.3-3-2- Total Cholesterol Measurement

Total cholesterol is measured after enzymatic hydrolysis and subsequent oxidation.

The indicator is quinoneimine. The amount of quinoneimine formed is proportional to the cholesterol concentration.

2.3-3-3- LDL and HDL Cholesterol Measurement

Low-density lipoproteins (LDL) in the sample are precipitated by the addition of phosphotungstic acid in the presence of magnesium ions. The supernatant obtained after centrifugation contains high-density lipoproteins (HDL), the cholesterol of which is measured using cholesterol oxidase. The reading is taken using a spectrophotometer at 500 nm.

3-Data Processing

Statistical analysis of the values and graphical representation of the data were performed using GraphPadPrism 8 software (San Diego, California, USA). Statistical differences between results were assessed using analysis of variance (ANOVA), followed by the Tukey-Kramer multiple comparison test, with a significance level of 5 %. All values are presented as mean ± SEM (standard error of the mean).

II- RESULTS

1- Acute Toxicity of the Aqueous Extract of *Citrus limon* (Rutaceae) Leaves

Gavage administration of a 2000 mg/kg body weight (BW) dose of the aqueous extract of *Citrus limon* (EACl) to mice resulted in no change in their behavior. However, the maximum dose of 5000 mg/kg BW administered to mice caused decreased motor activity, respiratory distress, and grouping in a corner of the cage during the first 30 minutes following treatment. After this time, their behavior returned to normal.

During 14 days of observation, no mouse mortality was recorded for the different doses (2000 and 5000 mg/kg BW) administered to the mice in the different groups.

2- Pharmacological Effects of Aqueous Extract *of Citrus limon* Leaves (EACl) on Obese Rats

2-1- Effects of EACl and Atorvastatin on Body Mass in Obese Rats

Body mass in healthy, non-obese control rats was 151.9 ± 0.7 g on day 0 (D_0) and did not change significantly ($p < 0.05$) from the beginning of the experiment (D_0) until day 28 (D_0) (Figure 1). In rats made obese, body mass increased significantly ($p < 0.001$), rising to 253.2 ± 0.9 g, and then did not change in obese control rats receiving the standard diet. However, in obese rats that continued to receive the high-fat, high-calorie diet, body mass increased progressively. Thus, on day 28, the body mass of these animals is 332.9 ± 0.3 g; an increase of 119.16 % compared to that of the control rats which received the standard diet.

In obese rats treated with atorvastatin and receiving the obesogenic diet for 21 days, body mass decreased significantly ($p < 0.05$), from 252.4 ± 0.6 g to 214.3 ± 0.5 g, and was then maintained ($p > 0.05$) until day 28 of the experiment. Similarly, in obese rats treated with EACl and receiving the obesogenic diet, body mass decreased significantly ($p < 0.05$) from day 21 onward. This decrease in body mass was dose-dependent, with masses of 237.3 ± 0.6 g and 208.7 ± 0.9 g, respectively, measured on day 28 in rats treated with EACl doses of 750 and 1000 mg/kg BW; i.e., decreases Significant ($p < 0.05$) increases of 28.71 % and 37.31 % ($p < 0.05$), respectively, compared to obese controls receiving the obesogenic diet. At this time, these animals had body masses that increased by 56.22 % and 37.39 % when treated with these two doses, compared to that of healthy rats.

2-2- Effects of EACl and Atorvastatin on Serum Total Cholesterol Concentration in Obese Rats

The results presented in Figure 2 indicate a 213.58 % increase ($p < 0.001$) in total cholesterol levels in obese rats (2.04 ± 0.02 g/L) compared to non-obese control rats (0.65 ± 0.01 g/L) at baseline (Day 0). Total cholesterol levels in healthy rats and obese rats receiving the standard diet (St) remained constant ($p > 0.05$) until Day 28 of the experiment.

In untreated obese rats that continued to receive the high-calorie, high-fat diet, cholesterol levels increased progressively and significantly ($p < 0.05$) from day 7 (2.45 ± 0.02 g/L) compared to obese control rats receiving the standard diet. By day 28, this total cholesterol level was 2.90 ± 0.01 g/L, representing a 42.16 % increase compared to obese control rats.

In rats treated with atorvastatin (10 mg/kg BW) and receiving a high-calorie, high-fat diet, total cholesterol levels decreased significantly ($p < 0.05$) as early as day 7 (1.35 ± 0.01 g/L), and from day 14 to day 28, total cholesterol levels became statistically identical ($p > 0.05$) to those of healthy (non-obese) control rats. In obese rats treated with the extract at doses of 750 and

1000 mg/kg BW, respectively, total cholesterol levels decreased in a dose-dependent and significant manner ($p < 0.05$) starting on day 7 (for the second dose) and day 14 (for the first dose). The dose of 1000 mg/kg BW brings the total cholesterol level back to normal, statistically identical to that of healthy rats ($p > 0.05$), from the 21st day, whereas on the 28th day this level is 1.03 ± 0.01 g/L in rats treated with the dose of 750 mg/kg BW; i.e. an increase of 58.46 % ($p < 0.01$) compared to that of healthy rats.

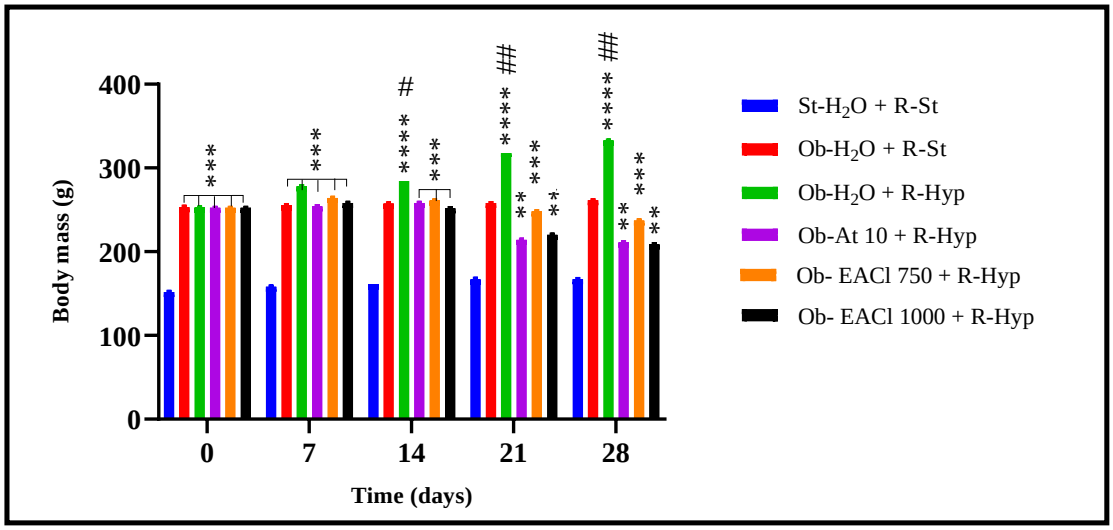

Figure 1: Change in body mass in rats treated with EACl or Atorvastatin for 28 days

n = 10

*** p < 0.01; *** p < 0.001 compared to healthy (non-obese) controls*

 # p < 0.05; ## p < 0.01 compared to obese controls

St-H_2O + R-St: Healthy (non-obese) control rats receiving the standard diet

Ob- H_2O + R-St: Obese control rats receiving the standard diet

Ob- H_2O + R-Hyp: Obese control rats receiving the high-calorie, high-fat diet

Ob-At 10 + R-Hyp: Obese rats treated with 10 mg/kg BW of Atorvastatin and receiving the high-calorie, high-fat diet

Ob-EACl 750 + R-Hyp: Obese rats treated with 750 mg/kg BW of EACl and receiving the high-calorie, high-fat diet

Ob-EACl 750 + R-Hyp: Obese rats treated with 1000 mg/kg BW of EACl and receiving the diet high in calories and fat

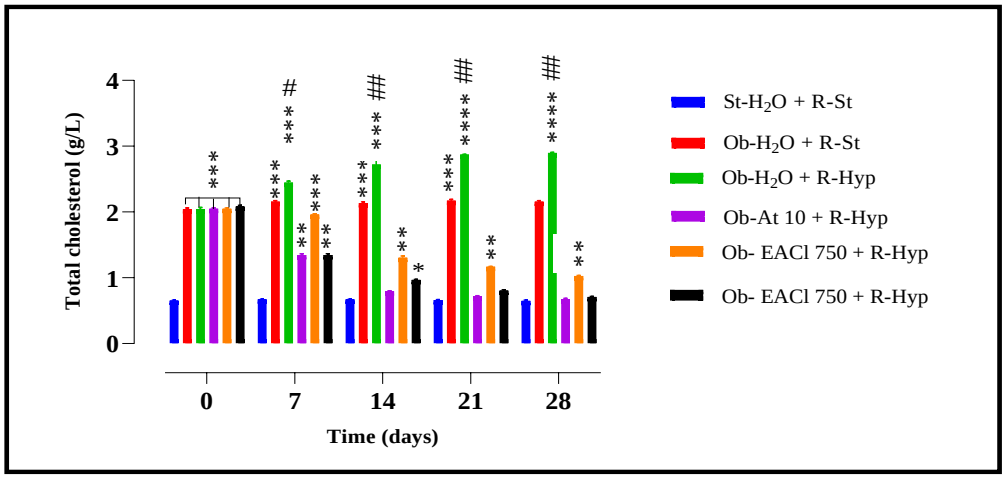

Figure 2: Change in serum total cholesterol concentration in rats treated with EACl or Atorvastatin for 28 days

n = 10

** p < 0.05; ** p < 0.01; *** p < 0.001 compared to healthy (non-obese) controls*

 # p < 0.05; ## p < 0.01 compared to obese controls

St-H_2O + R-St: Healthy (non-obese) control rats receiving the standard diet

Ob- H_2O + R-St: Obese control rats receiving the standard diet

Ob- H_2O + R-Hyp: Obese control rats receiving the high-calorie, high-fat diet

Ob-At 10 + R-Hyp: Obese rats treated with 10 mg/kg BW of Atorvastatin and receiving the high-calorie, high-fat diet

Ob-EACl 750 + R-Hyp: Obese rats treated with 750 mg/kg BW of EACl and receiving the high-calorie, high-fat diet

Ob-EACl 750 + R-Hyp: Obese rats treated with 1000 mg/kg BW of EACl and receiving the diet high in calories and fat

2-3- Effects of EACl and Atorvastatin on Serum HDL Cholesterol Concentration in Obese Rats

The HDL cholesterol level of non-obese control rats was 0.47 ± 0.01 g/L at the beginning of the experiment and did not change significantly ($p > 0.05$) until the end of the experiment (day 28) (Figure 3).

In rats made obese, the HDL cholesterol level decreased significantly ($p < 0.001$) compared to non-obese control rats. Its level dropped to 0.27 ± 0.01 g/L on day 0.

When the obese rats were not treated and received the standard diet, the HDL cholesterol level increased progressively and significantly, reaching 0.36 ± 0.01 g/L on day 28. The same is true for obese rats treated with EACl or Atorvastatin. In these cases, on day 28, their HDL cholesterol level is 0.38 ± 0.01 g/L in rats treated with EACl at 750 mg/kg BW and with Atorvastatin at 10 mg/kg BW. However, with a dose of 1000 mg/kg BW of EACl administered to obese rats, the HDL cholesterol level returns to normal on day 28 of treatment.

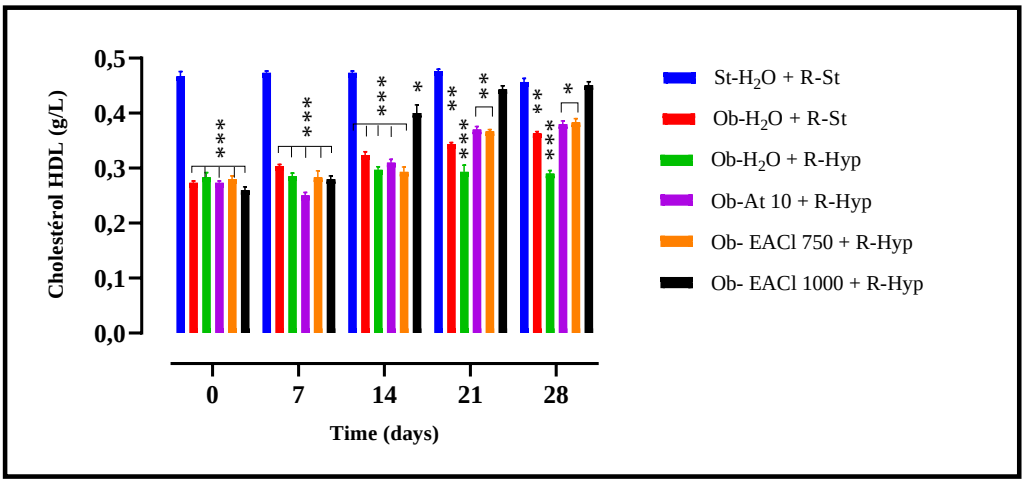

Figure 3: Change in serum HDL cholesterol concentration in rats treated with EACl or Atorvastatin for 28 days

n = 10

** p < 0.05; ** p < 0.01; *** p < 0.001 compared to healthy (non-obese) controls*

 # p < 0.05; ## p < 0.01 compared to obese controls

St-H_2O + R-St: Healthy (non-obese) control rats receiving the standard diet

Ob-H_2O + R-St: Obese control rats receiving the standard diet

Ob-H_2O + R-Hyp: Obese control rats receiving the high-calorie, high-fat diet

Ob-At 10 + R-Hyp: Obese rats treated with 10 mg/kg BW of Atorvastatin and receiving the high-calorie, high-fat diet

Ob-EACl 750 + R-Hyp: Obese rats treated with 750 mg/kg BW of EACl and receiving the high-calorie, high-fat diet

Ob-EACl 750 + R-Hyp: Obese rats treated with 1000 mg/kg BW of EACl and receiving the diet high in calories and fat

2-4- Effects of EACl and Atorvastatin on Serum LDL Cholesterol Concentration in Obese Rats

The LDL cholesterol level was 1.37 ± 0.01 g/L at baseline (Day 0) in non-obese control rats and varied non-significantly ($p > 0.05$) until Day 28 (Figure 4). When rats were made obese, the LDL cholesterol level increased significantly ($p < 0.01$), rising to 1.77 ± 0.01 g/L at baseline. When obese rats were not treated but received either the standard diet or the high-calorie, high-fat diet, the LDL cholesterol level increased, but not significantly ($p > 0.05$) until Day 28.

In contrast, in obese rats treated with EACl, LDL cholesterol levels decreased over time in a dose-dependent manner. Thus, on day 28, in rats treated with EACl at 1000 mg/kg BW, this level was virtually identical ($p > 0.05$) to that of healthy control rats, whereas it was 1.61 ± 0.01 g/L in rats receiving 750 mg/kg BW of EACl; representing an increase of 17.51 % ($p < 0.05$) compared to that of healthy rats. Similarly, in rats treated with atorvastatin and receiving a high-fat, high-calorie diet, LDL cholesterol levels decreased significantly from day 7 and returned to normal by day 28.

2-5- Effects of EACl and Atorvastatin on Serum Triglyceride Concentration in Obese Rats

The results presented in Figure 5 show a significant increase ($p < 0.001$) in triglyceride levels in obese rats compared to non-obese control rats; triglyceride levels were 1.34 ± 0.01 g/L and 0.71 ± 0.01 g/L, respectively, at the beginning of the experiment (Day 0). In healthy control rats or obese control rats receiving the standard diet, triglyceride levels did not change significantly ($p > 0.05$) until Day 28 of the experiment.

From day 14 onwards, in untreated obese rats receiving the standard diet, triglyceride levels decreased significantly ($p < 0.05$) until day 28 (1.29 ± 0.01 g/L). In contrast, in untreated obese rats receiving a high-calorie, high-fat diet, triglyceride levels (1.4 ± 0.05 g/L) increased significantly ($p < 0.001$) compared to obese control rats fed the standard diet. This increase raised

triglyceride levels to 1.92 ± 0.02 g/L on day 28 in untreated obese rats still receiving the high-calorie, high-fat diet. However, in obese rats treated with EACl at 750 and 1000 mg/kg BW or with Atorvastatin at 10 mg/kg BW, triglyceride levels decrease significantly ($p < 0.05$) from the 7th day and return these levels to normal by the 28th day of treatment.

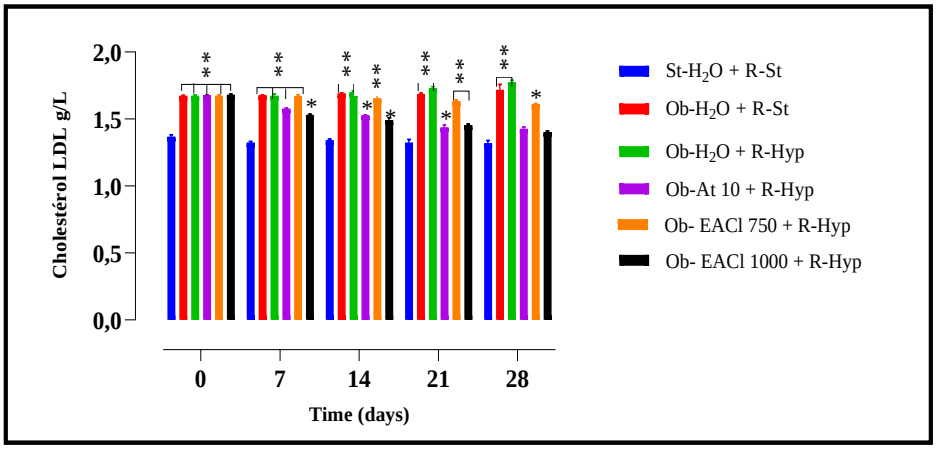

Figure 4: Change in serum LDL cholesterol concentration in rats treated with EACl or Atorvastatin for 28 days

n= 10

** p < 0.05; ** p < 0.01; *** p < 0.001 compared to healthy (non-obese) controls*

 # p < 0.05; ## p < 0.01 compared to obese controls

St-H2O + R-St: Healthy (non-obese) control rats receiving the standard diet

Ob-H2O + R-St: Obese control rats receiving the standard diet

Ob-H2O + R-Hyp: Obese control rats receiving the high-calorie, high-fat diet

Ob-At 10 + R-Hyp: Obese rats treated with 10 mg/kg BW of Atorvastatin and receiving the high-calorie, high-fat diet

Ob-EACl 750 + R-Hyp: Obese rats treated with 750 mg/kg BW of EACl and receiving the high-calorie, high-fat diet

Ob-EACl 750 + R-Hyp: Obese rats treated with 1000 mg/kg BW of EACl and receiving the diet high in calories and fat

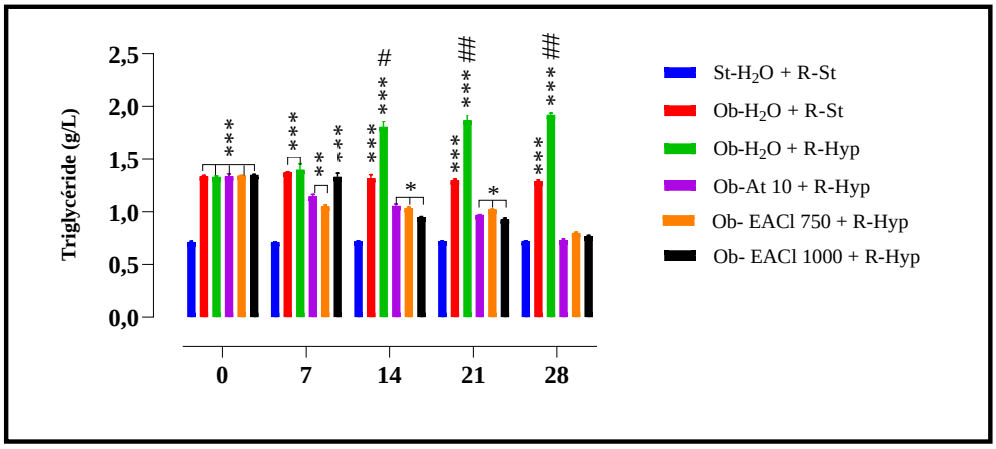

Figure 5: Variation in serum triglyceride concentration in rats treated with EACl or Atorvastatin for 28 days

n = 10

** p < 0.05; ** p < 0.01; *** p < 0.001 compared to healthy (non-obese) controls*

 # p < 0.05; ## p < 0.01 compared to obese controls

St-H2O + R-St: Healthy (non-obese) control rats receiving the standard diet

Ob-H2O + R-St: Obese control rats receiving the standard diet

Ob-H2O + R-Hyp: Obese control rats receiving the high-calorie, high-fat diet

Ob-At 10 + R-Hyp: Obese rats treated with 10 mg/kg BW of Atorvastatin and receiving the high-calorie, high-fat diet

Ob-EACl 750 + R-Hyp: Obese rats treated with 750 mg/kg BW of EACl and receiving the high-calorie, high-fat diet

Ob-EACl 750 + R-Hyp: Obese rats treated with 1000 mg/kg BW of EACl and receiving the diet high in calories and fat

III- DISCUSSION

An acute oral toxicity study of the aqueous extract of *Citrus limon* leaves (EACl) in female mice, conducted according to OECD 423 guidelines (OECD, 2001), showed that administration of EACl at doses of 2000 and 5000 mg/kg body weight (BW) did not cause any mortality in the treated mice after 14 days of observation. The fact that the maximum dose of 5000 mg/kg BW was not lethal to the mice indicates that the maximum tolerated dose (MTD) is higher than this dose,

and also that the 50 % lethal dose (LD_{50}) of this extract is well above 5000 mg/kg BW. According to Clarke and Clarke (1977), a plant extract administered orally is considered non-toxic in animal experiments when its LD50 is greater than 1000 mg/kg BW. Thus, EACl, whose LD_{50} is undeniably greater than 5000 mg/kg BW, is non-toxic when administered orally. This aqueous extract of *Citrus limon* leaves could therefore be used in pharmacology via this route of administration.

This lack of toxicity by gavage, observed with the aqueous extract of *Citrus limon* leaves, is also observed with other plant extracts from traditional African pharmacopoeia, such as the root bark of *Calotropis procera* (Apocynaceae), the leaves of *Moringa oleifera* (Moringaceae), and the leaves of *Lophira lanceolata* (Ochnaceae), according to the respective studies of Ouedraogo *et al.* (2013), Asiedu-Gyekye *et al.* (2014), and Oussou *et al.* (2016).

The study of the pharmacological effects of EACl on obesity, conducted on rats made obese by a high-calorie, high-fat diet, shows that the induced obesity manifests as excessive body weight gain that remains elevated throughout the 28-day experiment, compared to the weight of rats on a standard diet. Measurement of lipid parameters in these obese rats shows that this obesity is accompanied by elevated plasma levels of total cholesterol, LDL cholesterol, and triglycerides, with a decrease in HDL cholesterol. The hyperlipidemia observed in obese rats can be explained by the high lipid content of their diet, unlike the standard diet given to control rats. Several authors have shown that an increase in the lipid content of the ingested food leads to an increase in plasma cholesterol and LDL cholesterol concentrations and alters the composition of plasma lipoproteins, notably by increasing the proportion of cholesterol esters in VLDL and LDL. These changes in lipoprotein composition are associated with an increase in hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity, an enzyme involved in hepatic cholesterol synthesis, and in plasma acyl-CoA cholesterol acyltransferase (ACAT) activity (Fernandez *et al*., 1996).

When obese rats are treated with EACl at doses of 750 and 1000 mg/kg body weight, the lipid disturbances observed in the obese rats decrease, and these lipid parameters even become normalized. There is also a decrease in their body weight, which tends toward that of healthy control rats. The aqueous extract of *Citrus limon* leaves is therefore an anti-hyperlipidemic and anti-hypercholesterolemic substance and counteracts excessive weight gain in obese rats.The increase in HDL cholesterol levels in rats treated with EACl is similar to the results of the work by Changizi *et al.*, (2013), which showed that administration of *Portulaca oleracea* (Portulacaceae) extract at doses of 200, 400, and 800 mg/kg BW induces an increase in HDL cholesterol levels in rats fed a high-fat diet.

The effects of EACl at 1000 mg/kg BW are similar to those of atorvastatin, a reference anti-hypercholesterolemic drug. Indeed, according to Dean *et al*. (2002), atorvastatin at 10 mg induces a decrease in triglyceride, total cholesterol, and LDL cholesterol levels in dyslipidemic patients.

Atorvastatin is one of the classes of statins whose primary site of action is the liver, a target organ for cholesterol reduction. Statins work by competitively inhibiting HMG-CoA reductase, an enzyme that catalyzes the intracellular conversion of HMG-CoA to mevalonate, an early step in the biosynthesis of intracellular cholesterol in the body. This reaction limits endogenous cholesterol synthesis. Statins also increase the number of low-density lipoprotein (LDL) receptors on the surface of hepatocytes, thereby increasing LDL cholesterol uptake, enhancing its catabolism, and thus lowering plasma LDL cholesterol concentration (Allain, 2000). The increase in hepatic LDL receptors also leads to the inhibition of VLDL synthesis and, to a lesser extent, a reduction in the number of cholesterol-laden VLDL and LDL particles circulating in the blood. Through this mechanism, HMG-CoA reductase inhibitors lead to a decrease in triglycerides and a slight increase in HDL cholesterol (Zhang *et al.,* 2005).

The decrease in body mass in obese rats treated with EACl could be explained by the presence of saponins in this extract. Indeed, according to Han *et al.* (2000), saponins have anti-obesity properties.

The decrease in triglycerides in obese rats treated with EACl could be due to the presence of alkaloids in this extract. Some studies show that alkaloids can decrease triglyceride levels by increasing the expression of hepatic LDL receptors and inhibit lipid synthesis in human hepatocytes by activating AMPK (5'-Adenosine monophosphate-activated protein) (Brusq *et al.,* 2008).

The effects of EACl on induced obesity in rats justify the use of *Citrus limon* (Rutaceae) in traditional medicine in the treatment of this pathology.

IV-CONCLUSION

The acute toxicity study of EACl shows that this extract is non-toxic when administered orally. This oral route of administration is therefore recommended for the pharmacological use of this extract.

The study of the effects of EACl on obesity induced in rats by a high-calorie, high-fat diet shows that this extract leads to a decrease in body weight in obese rats and has dose-dependent anti-hyperlipidemic and anti-hypercholesterolemic effects in these treated obese animals. Furthermore, the effects of EACl (1000 mg/kg BW) on obesity are similar to those of atorvastatin (10 mg/kg BW), a reference anti-hypercholesterolemic substance. EACl could therefore

act against obesity through a mechanism similar to that of this statin.

These effects of EACl justify the use of *Citrus limon* (Rutaceae) in traditional medicine in the treatment of obesity.

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