

Anti-Obesity and Antihyperlipidemic Effects of *Elephantopus mollis* Kunth. Aqueous Extract on MACAPOS 2 Induced Obese Rats

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Abstract

Elephantopus mollis Kunth. (Asteraceae) is used in folk medicine by the populations of the Eastern region of Cameroon for its different therapeutic activities. The present study aimed to investigate the anti-obesity and antihyperlipidemic potential of *Elephantopus mollis* aqueous extract on MACAPOS (maize, cassava, palm oil, and sugar) 2 induced obesity in rats. Obesity was induced in 6- to 8-week-old *Wistar* rats with a local high-fat diet for 16 weeks. During 28 days, obese rats once a day, orally received *E. mollis* extract at different doses (50, 100, or 200 mg/kg body weight), or atorvastatin (reference drug at 10 mg/kg bw). During the treatment, body weight was recorded every week, and the food and water intakes every two days. At the end of treatment, animals were sacrificed under anaesthesia, after 12 hours of fasting. White adipose tissues (visceral, subcutaneous and peritesticular fats) and carcass were collected and weighted. Serum and liver were collected for biochemical estimations of lipid parameters. *E. mollis* aqueous extract at the doses used, significantly ($p < 0.01$) reduced body weight gain, fat tissues (−38.55% Em50; −38.39% Em100, or −37.07% Em200 for subcutaneous fat), serum lipids (LDL-cholesterol: −72.16% Em50, −79.43% Em100, and −87.08% Em200, total cholesterol: −61.38% Em50; −19.38% Em100; and −28.96% Em200 and triglycer-

ides: -41.94% Em50, -8.63% Em100, and -36.42% Em200) and hepatic lipids (triglycerides: -52.63% Em200 and total cholesterol: -25.13% Em50, -37.23% Em100, and -42.98% Em200) associated with an increase of HDL-cholesterol level (+92.04% Em100) compared to obese control rats. Improvement of lipid profile led to an important decrease of atherogenic index (+56.5% Em50 and +36.5% Em100). These results suggest that, *E. mollis* aqueous extract could have anti-obesity and antihyperlipidemic properties and improve arterial health, thus justifying its empirical use in the treatment of obesity.

Keywords

Elephantopus mollis, Obesity, Lipid Parameters, MACAPOS 2, High-Fat Diet

1. Introduction

The incidence of obesity, a serious public health problem, is rising worldwide in general, and particularly in urban settings of less developed and developing countries. Obesity represents an excess body fat accumulation resulting from an imbalance between regular energy intake and expenditure [1]. The high consumption of energy-dense diets, such as high-fat diets with reduced physical activity, has been pointed out as the prime cause of obesity [2]. In 2016, over 39% of the adults worldwide were overweight, among whom about one-third were obese. And according to the WHO, by 2030, 51% of the global population will be obese if nothing is done [3]. Obesity is increasing annually in Africa; the WHO reported that its prevalence increased from 12% in 2000 to 18.4% in 2021 in women, and from 4.1% to 7.8% in men during the same period [4]. In Cameroon, obesity prevalence increased from 4.9% (2009) to 9.5% (2016) [5].

Obesity is associated with various metabolic and phenotypic alterations, such as increased body weight, low-grade inflammation, insulin resistance, hyperinsulinemia, hyperleptinemia, hyperglycemia, hyperlipidemia, systemic inflammation, and hepatic steatosis [6]. Pharmacotherapy and lifestyle intervention are common choices for long-term weight reduction, but their effectiveness is usually compromised due to poor compliance. Pharmacotherapy is seriously challenged by the inevitable side effects [7]. In addition, continuous use of current medications may constitute an economic burden on the user [8]. Thus, intense efforts have been devoted to developing more tolerated anti-obesity food bioactives. Traditional anti-obesity plants might provide new oral anti-obesity compounds that can counter the high cost and poor availability of the current medicines for many rural populations in developing countries [9].

Elephantopus mollis Kunth. (Asteraceae) is a herbaceous perennial that originated from tropical America and was widely introduced to high rainfall tropical Africa, Asia, and the Pacific. This plant has been used in folk medicine as well as in Chinese traditional medicine for the treatment of hepatitis, tonsillitis, colds,

and carbuncles [10]. According to traditional healers in the East region of Cameroon, this plant is used to treat many diseases, including obesity, and to the best of our knowledge, no scientific study has been undertaken to verify these claims. This study, therefore, aimed at investigating the anti-obesity and anti-hyperlipidemic effect of *E. mollis* aqueous extract on MACAPOS 2-induced obesity in rats.

2. Materials and Methods

2.1. Plant Extract Preparation

The whole plant of *Elephantopus mollis* was collected in May 2022 at Diang (East region of Cameroon). Botanical identification was performed at the national herbarium, Yaoundé-Cameroon where the plant sample was compared to the voucher specimens N° 18231 SRF/cam. The sample was cleaned, sliced into small pieces, shade dried, and powdered with a grinder. Thereafter, the powder (300 g) was introduced into boiling water (3 L) for 5 hours. The mixture was filtered using Whatman paper N°3 and dehydrated to yield 31.5 g of dry dark aqueous extract.

Phytochemical screening of this extract for its active biological principles was conducted using the standard methods found in the literature.

2.2. Experimental Animals

Male albino *Wistar* rats (6 - 8 weeks old) were used in this experiment. They were raised under natural environmental conditions of light and temperature in the animal house of the Laboratory of Human Metabolism and Non-Communicable Diseases of the Institute of Medical Research and Medicinal Plants Studies (IMPM), Yaoundé, Cameroon. The animals were kept in polypropylene cages with a metal mesh cover at room temperature, with adequate ventilation, and then were allowed to acclimatize to laboratory environmental conditions for two weeks. Food and water were given *ad libitum*. Animal handling and experiments were performed according to the European Union directives on ethical evaluation of animal experiments [11] adopted by the Cameroon institutional national ethics committee, Ministry of Scientific Research and Innovation (N°: FWA-IRD 0001954).

2.3. Obesity Induction

All the ingredients of the diet were obtained from a local market in Yaoundé. Induction of obesity was done according to the method described by Kamgang *et al.* [12]. The high-fat diet was used to induce obesity in rats over 16 weeks [13]. In fact, after a two-week acclimatization period, rats were randomly subjected to a high-fat diet (Table 1). Food and water intakes were recorded every two days, and the weight variation was measured weekly during a 16-week period. To determine obese rats at the end of this treatment period, the Lee index (Li) was calculated using the body weight (bw in g) and naso-anal length (Lna in cm) as follows:

$$Li = \frac{\sqrt[3]{bw}}{Lna} \quad [14]$$

The animals with Lee index ≥ 0.31 were considered obese, selected, and randomly divided into 5 groups of five rats each for the next stage of the experiment.

Table 1. Diet composition per 1000 g [12].

Groups	Ingredients in g										
	Maize	Wheat	Stepped Cassava	Sucrose	Soya Bean	Fish Flour	Cabbage Palm	Palm Oil	Bones Flour	Vitamins Complex	Energy (kcal/kg)
ND	250	400	-	-	150	100	80	-	10	10	3400
HFD	80	110	220	50	280	30	-	200	20	10	4730

ND: normal diet; HFD: high-fat diet.

2.4. Experimental Design and Animal Treatment

The equivalent dose was calculated using the NOAEL calculation method [15] based on the dose administered to humans by traditional healers.

The rats were divided into six (6) groups of five animals each as follows:

- Normal control (NC) group: rats fed with a normal diet [12], receiving distilled water (10 mL/kg);
- Obese control (OC) group: obese rats fed with HFD, treated with distilled water (10 mL/kg);
- *Elephantopus mollis* (*Em*) treated groups: obese rats fed with HFD, treated with *E. mollis* extract, respectively, at 50, 100, or 200 mg/kg body weight (Em50, Em100, or Em200, respectively);
- Atorvastatin (AVAS) group: obese rats fed with HFD, treated with the reference drug atorvastatin at 10 mg/kg body weight.

Rats once daily received the respective treatments, which were administered by intra-gastric gavage for 28 consecutive days. During this period, the body weight was recorded every week, and the food and water intakes were recorded every two days. At the end of treatment, all rats were fasted overnight for 12 hours with free access to water, then sacrificed under anesthesia (diazepam, 10 mg/kg bw, and ketamine, 50 mg/kg bw). The blood was collected in dried tubes, centrifuged, and the serum obtained was used to determine the serum lipid profile. White adipose tissues (visceral, subcutaneous, and testicular fats), carcass, and the liver were collected and weighed. The liver was immediately washed with an ice-cold saline solution (NaCl 0.9%), and a portion (200 mg) was homogenized in 1 mL of Tris-HCl (0.2 M, pH 7.4) buffer solution, then centrifuged (2500 g, 25 min). The supernatant obtained was used for biochemical estimations of total cholesterol and triglycerides.

Biochemical Analysis: Determination of Lipid Parameter Content

Total cholesterol (TC), HDL-cholesterol (HDL-C), and triglycerides (TG) were assayed through colorimetric methods with commercially available test kits according to the manufacturer's recommendations (Lab Kit brand).

LDL-C (Low-density lipoprotein-cholesterol) was calculated with the standard

formula [16]:

$$\text{LDL-C} = \text{TC} - \left(\text{HDL-C} + \frac{\text{TG}}{5} \right)$$

The atherogenic index (AI) was calculated as follows [17]:

$$\text{AI} = \text{TC} - \frac{\text{HDL-C}}{\text{TC}}$$

2.5. Statistical Analysis

The results were expressed as mean \pm standard error of the mean. The statistical analyses were performed by one-way analysis of variance (ANOVA) associated with Tukey's test, followed by the Dunnett test for non-repeated measures. Two-way ANOVA was used, followed by Bonferroni's multiple comparisons test, to compare body weight, food and water intakes. GraphPad Prism 8.0.1 was used for analyses. The difference between and within various groups was significant at $p < 0.05$.

3. Results

3.1. Phytochemical Screening of *E. mollis* Aqueous Extract

The phytochemical screening of *E. mollis* aqueous extract revealed the presence of different classes of chemical compounds such as phenols, tannins, anthraquinones, saponins, sterols, flavonoids, anthocyanidins, coumarins, triterpenes, alkaloids, and polysaccharides.

3.2. Effect of *E. mollis* Aqueous Extract on Food and Water Intake of Obese Rats

During the treatment, food and water intake of obese control rats (OC) did not significantly vary compared to normal control rats (NC) (Figure 1). The administration of *E. mollis* aqueous extract decreased the food and water intakes. From the 21st day of the treatment, the extract at 100 mg/kg bw significantly ($p < 0.05$) decreased food intake compared to OC (Figure 1(a)). This dose of extract at the same period also significantly decreased water intake compared to OC (Figure 1(b)).

3.3. Effect of *E. mollis* Aqueous Extract on Body Weight Variation of Obese Rats

Body weight of obese control rats (OC) remained significantly ($p < 0.01$) high during the treatment (+31.6% at day 28) compared to normal control rats (NC). *E. mollis* aqueous extract at 200 mg/kg bw, as well as atorvastatin, slightly decreased the body weight from the 14th day of treatment (Figure 2, Table 2).

3.4. Effects of *E. mollis* Aqueous Extract on Adipose Tissue Development and Carcass in Obese Rats

The MACAPOS 2 high-fat diet significantly ($p < 0.01$) increased visceral (+197.19%),

peri-testicular (+90.60%), and subcutaneous (+333.57%) white adipose tissues in non-treated obese rats compared to the normal control group (NC). Administration of *E. mollis* aqueous extract moderately decreased visceral (−4.87% Em50, −16.04% Em100, or −12.58% Em200) and testicular fat (−3.86% Em50, −13.68% Em100, or −2.46% Em200). The plant extract at different doses, as well as the reference drug, remarkably ($p < 0.01$) reduced subcutaneous fat (−38.55% Em50; −38.39% Em100, or −37.07% Em200) compared to obese control rats (Table 2).

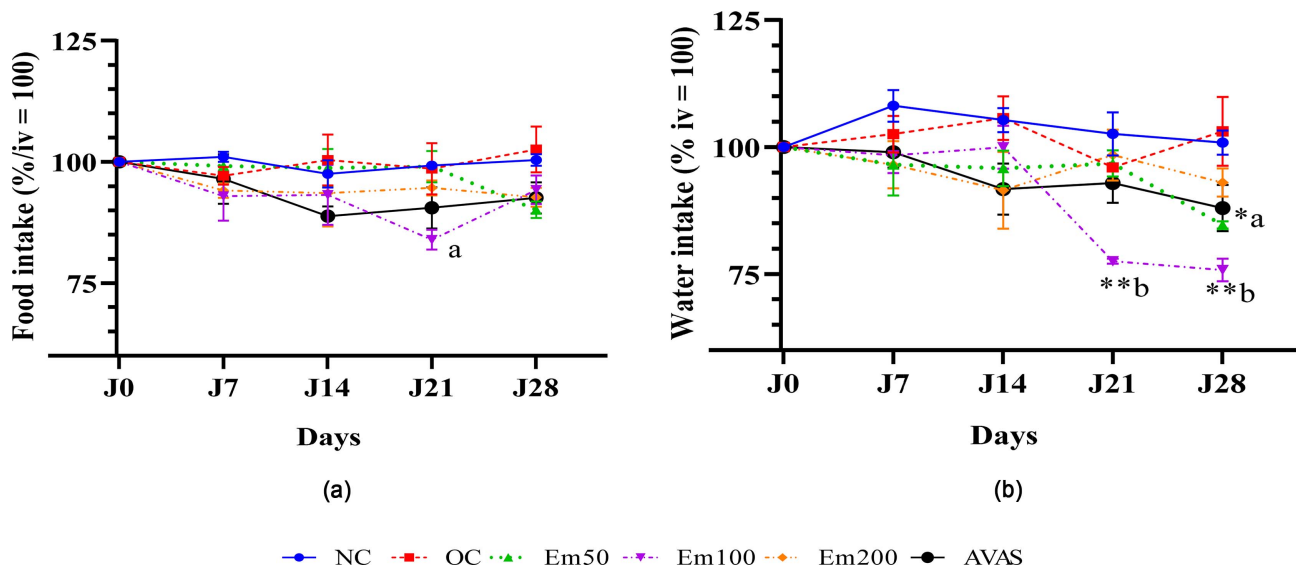


Figure 1. Effects of *E. mollis* aqueous extract, atorvastatin, or distilled water on food (a) and water (b) intakes (expressed as % compared to initial values) of high-fat diet-induced obese rats during 28 days of treatment. NC: normal control rats; OC: obese control rats; Em50, Em100, Em200: obese rats treated with *E. mollis* extract, respectively, at 50, 100, or 200 mg/kg bw; AVAS: obese rats treated with atorvastatin 10 mg/kg bw. Significant difference: * $p < 0.05$, ** $p < 0.01$ compared to NC; ^a $p < 0.05$, ^b $p < 0.01$ compared to OC; $n = 5$.

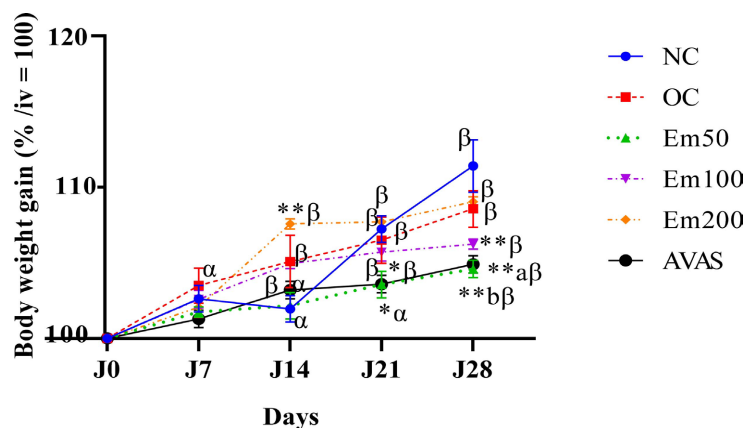


Figure 2. Body weight variation (expressed as % of initial values) of obese rats during 28 days of treatment. NC: normal control rats; OC: obese control rats; Em50, Em100, Em200: obese rats treated with *E. mollis* extract, respectively, at 50, 100, or 200 mg/kg bw; AVAS: obese rats treated with atorvastatin 10 mg/kg bw. Significant difference: ^α $p < 0.05$; ^β $p < 0.01$ compared to initial value; * $p < 0.05$, ** $p < 0.01$ compared to NC; ^a $p < 0.05$, ^b $p < 0.01$ compared to OC; $n = 5$.

Table 2. Body weight (expressed in g) of obese rats during 28 days of treatment.

	D0	D7	D14	D21	D28
NC	262.5 ± 11.1	268.5 ± 9.8	268.5 ± 12.9	280.5 ± 9.7	292.3 ± 6.0
OC	356.4 ± 8.0**	369 ± 7.7**	374.6 ± 8.7**	379.6 ± 8.4**	384.6 ± 9.6**
Em50	338.3 ± 5.8**	345.3 ± 5.2**	350.0 ± 5.4**	351.7 ± 5.2**	353.7 ± 5.8**
Em100	333.0 ± 8.4**	341.8 ± 11.0**	350.8 ± 14.2**	352 ± 21.9**	352.5 ± 26.0**
Em200	337.0 ± 19.0**	341.8 ± 18.7**	363.7 ± 3.3**	363.0 ± 4.8**	366.3 ± 3.3**
AVAS	351.7 ± 13.1**	356.3 ± 15.4**	363 ± 17.3**	364.3 ± 18.4**	369.0 ± 21.1**

NC: normal control rats; OC: obese control rats; Em50, Em100, Em200: obese rats treated with *E. mollis* extract, respectively, at 50, 100, or 200 mg/kg bw; AVAS: obese rats treated with atorvastatin 10 mg/kg bw. Significant difference: *p < 0.05, **p < 0.01 compared to NC; n = 5.

The carcass weight of obese control rats (OC) significantly (p < 0.01) decreased compared to NC. Just like atorvastatin, plant extract at the doses used slightly increased the relative carcass weight (+4.82% Em50, +5.37% Em100, and +3.40% Em200) at the end of treatment compared to OC (Table 3).

Table 3. Relative weight of visceral fat, peri-testicular fat, subcutaneous fat, and carcass (expressed in %) of obese rats after 28-day treatment.

	NC	OC	Em50	Em100	Em200	AVAS
Visceral Fat	2.14 ± 0.32	6.36 ± 0.19**	6.05 ± 0.35**	5.34 ± 0.42**	5.56 ± 0.18**	5.60 ± 0.50**
Peritesticular Fat	1.50 ± 0.11	2.85 ± 0.18**	2.74 ± 0.29**	2.46 ± 0.14**	2.78 ± 0.17**	2.67 ± 0.30**
Subcutaneous Fat	1.40 ± 0.06	6.07 ± 0.27**	3.73 ± 0.23** ^b	3.74 ± 0.34** ^b	3.82 ± 0.19** ^b	3.59 ± 0.23** ^b
Carcass	74.59 ± 1.22	67.42 ± 1.25**	70.67 ± 0.52	71.04 ± 1.17	69.71 ± 0.29*	70.95 ± 1.02

NC: normal control rats; OC: obese control rats; Em50, Em100, Em200: obese rats treated with *E. mollis* extract, respectively, at 50, 100, or 200 mg/kg bw; AVAS: obese rats treated with atorvastatin 10 mg/kg bw. Significant difference: *p < 0.05, **p < 0.01 compared to NC; ^bp < 0.01 compared to OC; n = 5.

3.5. Effect of *E. mollis* Aqueous Extract on Lipemia and Atherogenic Index of Obese Rats

High-fat diet significantly (p < 0.01) increased total cholesterol, triglycerides, and LDL-cholesterol (+115.29%, +44.77% and 592.47%, respectively) while decreasing HDL-cholesterol (−30.82 %) in obese control rats (OC) compared to normal control rats (Figure 3). Administration of *E. mollis* aqueous extract remarkably (p < 0.01) decreased the serum level of total cholesterol (−61.38% Em50; −19.38% Em100; and −28.96% Em200) in obese rats compared to OC (Figure 3(a)). Better than atorvastatin, the plant extract at the used doses significantly (p < 0.01) reduced serum triglycerides (−41.94% Em50, −8.63% Em100, and −36.42% Em200) compared to OC, bringing them to a level comparable to that of the normal control rats (NC) (Figure 3(b)). In a dose-dependent manner, plant extract led to a significant (p < 0.01) decrease in serum LDL-cholesterol level (−72.16% Em50,

–79.43% Em100, and –87.08% Em200) compared to obese control rats, bringing it to a level comparable to that of the normal control rats (**Figure 3(c)**). The plant extract at 100 mg/kg bw increased ($p < 0.01$) serum level of HDL-cholesterol (+92.04%) compared to OC (**Figure 3(d)**).

The atherogenic index remarkably ($p < 0.01$) increased (+111%) in obese control rats compared to normal control rats. Better than atorvastatin, *E. mollis* aqueous extract, respectively, at 50 and 100 mg/kg bw, led to a remarkable ($p < 0.01$) decrease in the atherogenic index: +56.5% and +36.5%, bringing it into the normal range (**Figure 3(e)**).

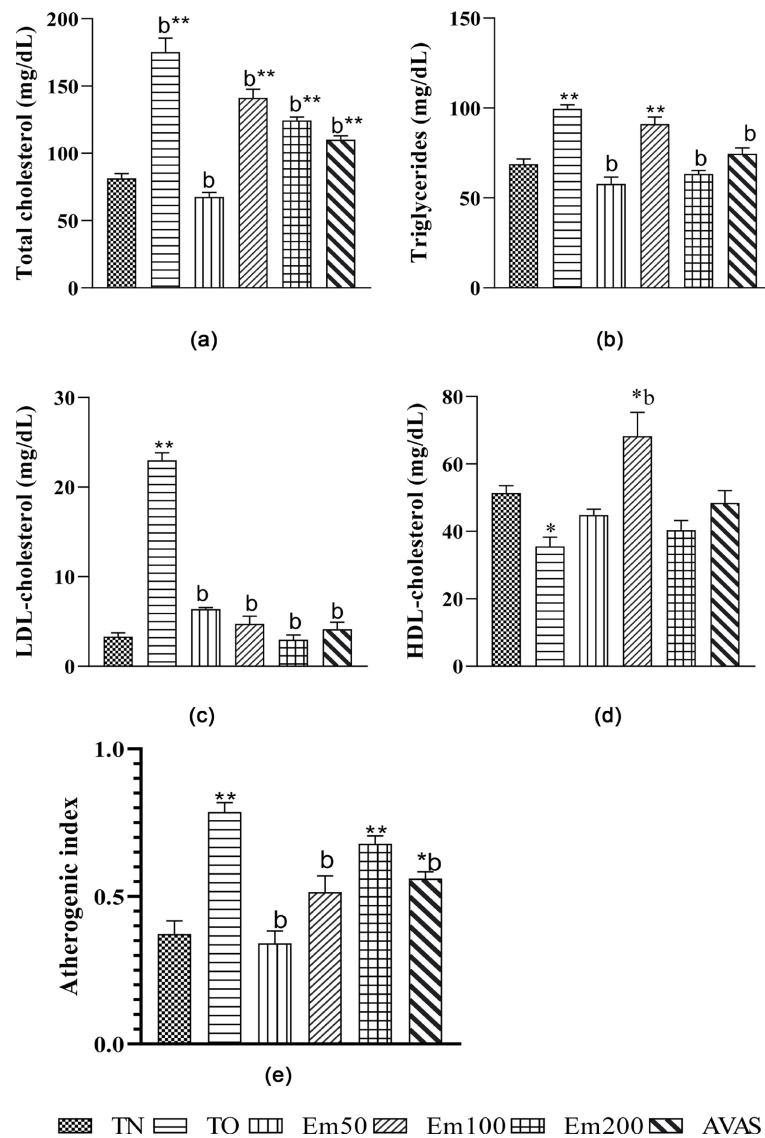


Figure 3. Serum lipid: total cholesterol (a), triglycerides (b), LDL-cholesterol (c), HDL-cholesterol (d), and atherogenic index (e) of obese rats after 28 days of once daily treatment. NC: normal control rats; OC: obese control rats; Em50, Em100, Em200: obese rats treated with *E. mollis* extract, respectively, at 50, 100, or 200 mg/kg bw; AVAS: obese rats treated with atorvastatin 10 mg/kg bw. Significant difference: * $p < 0.05$, ** $p < 0.01$ compared to NC; ^a $p < 0.05$, ^b $p < 0.01$ compared to OC; $n = 5$.

3.6. Effect of *E. mollis* Aqueous Extract on Hepatic Lipid Profile in Obese Rats

A high-fat diet significantly ($p < 0.01$) increased hepatic lipids, including total cholesterol and triglycerides, compared to normal control rats (NC). *E. mollis* aqueous extract at 200 mg/kg bw significantly ($p < 0.01$) decreased hepatic triglycerides (-52.63%) compared to obese control rats (Figure 4(a)). The extract also, in a dose-dependent manner, remarkably ($p < 0.01$) lowered hepatic total cholesterol, respectively: -25.13% (Em50), -37.23% (Em100), or -42.98% (Em200) to a level similar to that of the normal control rats (Figure 4(b)).

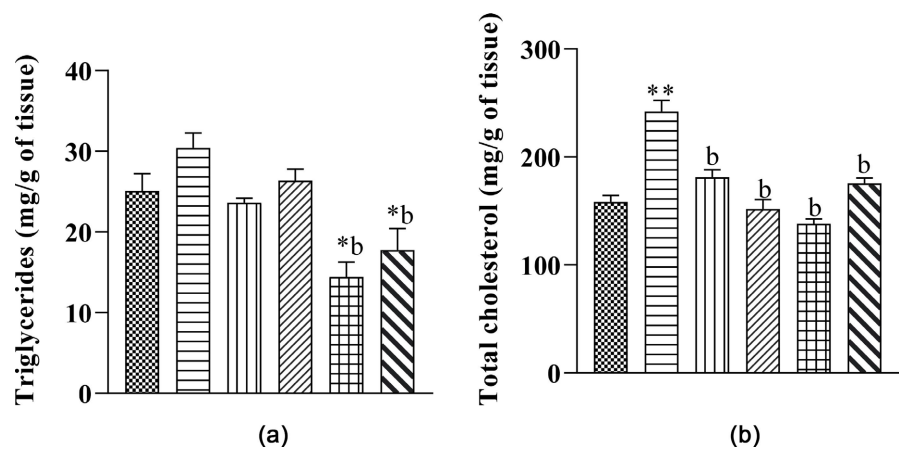


Figure 4. Hepatic lipid profile: triglycerides (a) and total cholesterol (b) of rats after 28 days of once daily treatment. NC: normal control rats; OC: obese control rats; Em50, Em100, Em200: obese rats treated with *E. mollis* extract, respectively, at 50, 100, or 200 mg/kg bw; AVAS: obese rats treated with atorvastatin 10 mg/kg bw. Significant difference: * $p < 0.05$, ** $p < 0.01$ compared to NC; ^a $p < 0.05$, ^b $p < 0.01$ compared to OC; $n = 5$.

4. Discussion

The present study aimed at investigating the anti-obesity and antidyslipidemic potential of *E. mollis* aqueous extract in obese rats MACAPOS 2. The MACAPOS 2 high-fat diet, inspired by the Cameroon western region local diet, induced visceral obesity characterized by fat accumulation, dyslipidemia, hepatic steatosis, and low lean mass. Previous studies showed that feeding animals with an energy-dense food, such as a high-fat diet (HFD), represents the most effective technique to induce an animal model of obesity close to that of humans [18] [19]. In general, long-term intake of high-energy foods will increase the risk of obesity and other metabolic diseases. Overfeeding is thought to be the main mechanism responsible for HFD-induced obesity and fat deposits [20].

Obesity is characterized by body weight gain and fat accumulation, associated with lipid and glucose metabolism disorders [21]. The current study showed that *E. mollis* aqueous extract reduced body weight and fat accumulation. The observed decrease in body weight could be associated with the remarkable decrease in white adipose tissues (visceral, peri-testicular, and subcutaneous) and not with the lean mass represented by the increase in carcass weight observed in animals

treated with plant infusion at the end of treatment. Moreover, the decrease in fat accumulation was associated with a reduction in food intake. The anti-obesity potential of the *E. mollis* extract could be attributed to its phytochemical constituents, including flavonoids, saponins, steroids, alkaloids, and triterpenes, which are known as lipid-lowering compounds. Indeed, flavonoids directly stimulate the breakdown of triglycerides into free fatty acids in visceral fat deposits and act on the regulatory pathways of lipid metabolism by inhibiting lipogenesis [22].

Obese individuals have a high potential of developing dyslipidemia and cardiovascular disease [23]. Abnormal lipid metabolism is generally associated with increased total cholesterol and decreased HDL-cholesterol in the serum [24]. The use of inhibitors of HMG-CoA reductase (the enzyme that converts HMG-CoA into mevalonic acid, a cholesterol precursor), such as atorvastatin, is one of the therapeutic approaches to treat dyslipidemia and hypercholesterolemia. Atorvastatin has the liver as its target organ. It inhibits hepatic synthesis of apolipoprotein B100, determining a reduction of the synthesis and secretion of triglyceride-rich lipoproteins [25]. Administration of *E. mollis* aqueous extract, as well as atorvastatin (lipase inhibitor) to obese rats, remarkably reduced serum levels of total cholesterol, triglycerides, and LDL-cholesterol compared to obese control rats. This result suggests that the *E. mollis* infusion may act like atorvastatin by inhibiting cholesterol synthesis [26]. This hypolipidemic potential of *E. mollis* extract on serum lipids may be due to the individual or combined effect of its secondary metabolites, including flavonoids, alkaloids, saponins, triterpenes, sterols, and tannins, which are known for their lipid-lowering effects by reducing cholesterol and triglyceride levels in rats [27]. Unlike atorvastatin, the plant extract at 100 mg/kg bw remarkably increased serum HDL-cholesterol levels at the end of treatment compared to obese control animals. This result suggests that the extract may also act through a different pathway than atorvastatin. This lipid-lowering activity could be a consequence of the significant reduction in fat tissues observed in the animals. The improvement of serum lipid profile led to a remarkable decrease of atherogenic index. Thus, suggesting an improvement of arterial health by the plant material. The increase in serum HDL-cholesterol is strongly associated with decreased risk of cardiovascular diseases. Indeed, it is well known that the decrease of LDL-cholesterol and the increase in HDL-cholesterol lead to an acceleration of the intake of cholesterol deposits to the liver for catabolism and excretion. On the other hand, an increase in HDL-cholesterol is involved in preventing the oxidation of LDL-cholesterol [28]. The improvement in arterial health with the aqueous extract of *E. mollis* could also be linked to the presence in this extract of sterols that inhibit intestinal absorption of cholesterol and lower serum LDL-cholesterol levels, thus preventing the formation of atherosclerotic plaques [29].

Obesity leads to hepatic morphological changes and hepatic steatosis [30]. Indeed, excessive accumulation of fat in obesity limits the storage capacity of the adipose tissue and increases the accumulation of fat in other tissues, including the liver, muscle, and heart [30] [31]. The MACAPOS 2 high-fat diet led to hepatic

steatosis with increased total cholesterol and triglycerides in the liver of obese rats. Administration of *E. mollis* aqueous extract significantly decreased hepatic total cholesterol and triglycerides levels, with a much more pronounced activity at 200 mg/kg bw. This result suggests that the extract may act against hepatic steatosis by reducing fat accumulation in the liver. This beneficial effect of *E. mollis* extract on liver lipids could be due to the isolated or combined action of its phytochemical lipid-lowering compounds, such as saponins, flavonoids, polyphenols, and anthocyanidins [32].

5. Conclusion

The results of this study showed that *E. mollis* aqueous extract possesses potent anti-obesity and antidyslipidemic properties, improves arterial health, and acts against non-alcoholic hepatic steatosis in MACAPOS 2 induced obese rats. This may justify the use of this plant by the populations of the eastern region of Cameroon in the treatment of obesity. However, it lacks the HPLC profile of this extract due to the current condition limit, which may contribute to the quality control for the extract. The molecular mechanism of the anti-obesity and antidyslipidemic potential of *E. mollis* aqueous extract has not been elucidated.

Data Availability

The data used to support the findings of this study are included within the article.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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