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In Vitro Antioxidant Potential and Effect of Elephantopus Mollis Kunth. Aqueous Extract on Glycemia Reactivity and Anthropometric Parameters in MACAPOS 2 High-Fat Diet Fed Rats

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ABSTRACT

The nutritional transition in sub-Saharan Africa, marked by increased consumption of energy-dense foods, has contributed to the rising prevalence of type 2 diabetes. Oxidative stress plays a central role in insulin resistance and glucose intolerance. The validation of traditional medicinal plants such as Elephantopus mollis (Asteraceae) may provide alternative therapeutic strategies. Elephantopus mollis is used in Cameroon eastern region folk medicine in the management of diabetes and obesity. This study aimed to evaluate the phytochemical profile, in vitro antioxidant activity, and the effect of E. mollis aqueous extract on glycaemia reactivity and anthropometrics parameters on MACAPOS 2 high-fat diet fed rats. Total phenolic, flavonoids, tannins, alkaloids and saponins contents of extract were determined using standard methods. The Antioxidant potential was explored considering 1.1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, nitric oxide radical inhibiting activity, Ferric Reducing Antioxidant Power (FRAP) ability, and total antioxidant capacity of the extract. Thirty-six male Wistar rats were fed a standard or high-fat diet for 30 days and treated concomitantly with once daily oral administration of E. mollis aqueous extract (Em50, 100 and 200 mg/kg b.w.) or metformin (70 mg/kg b.w.). The effect of E. mollis were investigated on, fasting glycaemia, glucose tolerance, insulin sensitivity, body weight gain, white adipose tissues (visceral, perirenal and peritesticular fats), food and water intakes. In Elephantopus mollis aqueous extract, saponins were the most abundant quantified metabolites (907.5 ± 18.37 µg SE/g), followed respectively by total polyphenols (788.67 ± 11.58 µg GAE/g), flavonoids (189.02 ± 7.96 µg QE/g), alkaloids (108,52 ± 9,75 µg QiE/g) and tannins (5,25 ± 0,18 µg TAE/g). This extract showed strong antioxidant activity by inhibiting nitric oxide, scavenging DPPH free radicals, reducing power. The MACAPOS 2 high-fat diet increased fasting glycemia, induced glucose tolerance, insulin resistance, and fat accumulation. The E. mollis extract decreased fasting glycemia, body weight gain, food and water intake. This extract remarkably ($p < 0.01$) decreased white adipose tissues associated with a significant improvement of glucose tolerance, and peripheral insulin sensitivity. E. mollis aqueous extract exhibited significant antioxidant potential, decreased fasting glycemia, improved glucose tolerance, insulin sensitivity and anthropometric parameters thus justifying its empirical use in the management of diabetes and obesity.

KEYWORDS: Elephantopus mollis, MACAPOS 2, high-fat diet, glycemia, antioxidant potential.

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1. INTRODUCTION

diabetes mellitus has emerged as a preeminent global public health challenge. Its global prevalence is increasing at an alarming rate and the developing countries are the most affected. In 2021, the global prevalence of diabetes worldwide was 537 million people. This number is expected to increase to 784 million in 2045 (IDF, 2021). The increase of diabetes prevalence in sub-saharan africa is correlated to the rapid uncontrolled urbanization and various lifestyle modifications including sedentary lifestyle and overconsumption of saturated fats and refined sugars (Banday et al., 2020). This lifestyle change leads to fat accumulation, glucose tolerance and peripheral insulin resistance (Buettner et al., 2007). If left unmanaged, this metabolic state inevitably progresses to chronic hyperglycemia, thus, type 2 diabetes mellitus. Type 2 diabetes constitutes about 90–95% of all type of diabetes and is mainly characterized by peripheral insulin resistance and β-cell dysfunction (Banday et al., 2020; DeFronzo et al., 2015). This pathologic condition is associated with the excessive generation of reactive oxygen species (ROS), which induce oxidative damage to biomolecules, thereby exacerbating tissue injury and promoting macro- and microvascular complications (Giacco & Brownlee, 2010).

As the knowledge of diabetes heterogeneity increases, more appropriate therapy is needed. Glycemic control associated with fat decrease and antioxidant activity may be the benefic approach to regress diabetes. Despite the availability of conventional pharmacotherapies, their long-term efficacy is often hampered by adverse side effects. In addition, continuous use of current medications may constitute an economic burden on the user particularly in sub-Saharan Africa (Atun et al., 2017). Consequently, there is an urgent need to explore natural bioactive substances as alternative or complementary therapeutic strategies (Eddouks et al., 2014). Elephantopus mollis Kunth. (Asteraceae) holds a prominent position in tropical African ethnomedicine for the management of metabolic and inflammatory disorders (Tsabang et al., 2016). According to traditional medicine practitioners in the Eastern Region of Cameroon, this plant is largely used in the treatment of many diseases including diabetes mellitus and obesity. The present study aimed to evaluate the phytochemical profile, in vitro antioxidant activity, and the effect of E. mollis aqueous extract on glycaemia reactivity and anthropometrics parameters on MACAPOS 2 high-fat diet fed rats.

2. MATERIALS AND METHODS

Plant Material and Extraction

Fresh Elephantopus mollis Kunth. plant, was harvested in Diang locality, Lom and Djerem Division (Easte Region of Cameroon), during the month of May 2022. Botanical identification was conducted at the Yaounde-Cameroon National Herbarium in comparison to the material of MEZILI P. n°140 with the voucher specimen n°18231 SRF/Cam. The whole plant was cleaned, sliced into small pieces, shade dried and powdered. To simulate traditional ethnomedicinal preparation, Elephantopus mollis aqueous extract (an infusion) was prepared by adding 300 g of the plant powder to 3000 mL of boiling water for 5 hours. The mixture was filtered through Whatman No. 3 paper and dehydrated to yield 31.5 g of dry dark aqueous extract.

Phytochemical Analysis

The active biological principles of this extract were identified using the standard qualitative screening methods (Odebiyi et Sofowara, 1978; Aziz-ur-Rehmana et al., 2011). Quantitative determination of major secondary metabolites was performed using spectrophotometric and gravimetric methods.

Total polyphenols content

Total polyphenols content (TPC) was determined using the Folin–Ciocalteu method described by Singleton and Rossi (1965). Briefly, 0.1 mL of each extract (4 mg/mL) was mixed with 0.75 mL of Folin-ciocalteu reagent (10-fold dilution). The entire mixture was incubated at room temperature (25 ± 2°C). Five (5) min later, 0.75 mL of a sodium carbonate solution (Na_2CO_3, 6%) was added. The mixture was homogenized and incubated for 90 min at room temperature (25 ± 2°C; in the dark), and then read the absorbance at 725 nm against a reagent blank. Gallic acid (0-1000 µg/mL) was used as a reference. Results were expressed as micrograms of gallic acid equivalents per gram of dry matter (mg GAE/g DM). Each test was performed in triplicate.

Flavonoids content

Total flavonoid content was assessed using the aluminum chloride colorimetric method (Zhishen et al., 1999). Briefly, 0.5 mL of each extract (4 mg/mL) was added to 1.5 mL of methanol, subsequently 0.1 mL of aluminum chloride ($AlCl_3$, 10%), 0.1 mL of potassium acetate (CH_3COOK, 1M), and 2.8 mL of distilled water were added. The mixture was well homogenized and incubated for 30 min at room temperature (25 ± 2 °C) and the absorbance was read at 415 nm wavelength against the reagent blank. Quercetin (0-1000 µg/mL) was used as a reference. Results expressed as micrograms of quercetin equivalent per gram of dry matter (mg QE/g DM). Each test was performed in triplicate.

Tannins content

The method of Bainbridge et al., (1996) was used to determine the total tannins content of this extract. Briefly, 1 mL of each extract (4 mg/mL) was mixed with 5 mL of working solution [50 g vanillin + 4 mL HCl (1N) in 100 mL distilled water], and then the mixture was incubated at 30 °C for 20 min. The absorbance was read at the wavelength of 500 nm against the blank (without extract). Tannic acid (0-1000 µg/mL) was used to establish the calibration range and tannin content. Results were expressed as micrograms tannic acid

equivalent per gram of dry matter (mg TAE/g DM). Each test was performed in triplicate.

Alkaloids content

Total alkaloids and saponins were quantified using gravimetric methods according to Harborne (1973).

Briefly, 100 mg of each extract was subjected to extraction in 10 mL of ethanol solution (80%). The whole was well homogenized and centrifuged at a speed of 5000 rpm for 10 min. After centrifugation, 1 mL of the supernatant of each extract was taken and introduced into a test tube, followed by the respective addition of 1 mL of acidified $FeCl_3$ (0.025 M) solution (0.5 M HCl) and 1 mL of an ethanolic solution of 1,10-phenanthroline (0.05 M). The whole mixture was again incubated at 100 °C in a water bath for 30 min. The absorbance of the reddish complex formed was read at the wavelength of 510 nm against the blank. Quinine at the concentration of 25 µg/mL was used as the primary standard and the alkaloid content was expressed as micrograms of quinine equivalent per gram of dry matter (mg QiE/g DM). Each test was performed in triplicate.

Saponins content

The determination of total saponins in E. mollis aqueous extract was performed using the method described by Hiai et al. (1976). Two hundred (200) µL of plant extract was introduced into a test tube and then, 200 µL of alcoholic vanillin solution (prepared in 80% ethanol) and 2000 µL of sulfuric acid solution (72%) were added to it. The mixture was homogenized and placed in a water bath at 60°C for 10 minutes. The absorbance of this prepared solution was read after incubation at wavelength of 535 nm against the blank. A saponins standard was used at different concentrations (0-1000 µg/mL) to establish the calibration range. Results were expressed as micrograms of saponins equivalent per gram of sample dry matter (mg SaE/g DM). Each test was performed in triplicate.

In Vitro Antioxidant assays

DPPH free radical scavenging activity

The DPPH (1.1-Diphenyl-2-picrylhydrazyl) free radical scavenging activity of the extract was assessed according to (Sanchez-Moreno et al.1998). 0.1 mM DPPH solution (2 mL) was mixed with 20 µL of various concentrations (0.5-4 mg/mL) of extract dissolved in 95% methanol. The mixture was incubated for 30 minutes in the dark and absorbance was measured at 517 nm. DPPH inhibition rates (I) and the IC_{50} (concentration required to inhibit 50% of radicals) were determined. The IC_{50} values were determined using nonlinear regression analysis (log[inhibitor] vs. normalized response) through GraphPad Prism 8.0.1.

$I (\%) = ((A0 - As)/ A0) \times 100$

Nitric oxide radical inhibiting activity

The inhibiting activity of E. mollis aqueous extract (0.5-4 mg/mL) on the nitric oxide radical was measured using the sodium nitroprusside and Greiss reagent (Ebrahimzadeh et al., 2010). For radical scavenging assays, percentage inhibition was plotted against extract concentrations to generate dose–response curves. The half-maximal inhibitory concentration (IC_{50}) values were calculated using nonlinear regression analysis. The IC_{50} values were determined using nonlinear regression analysis (log[inhibitor] vs. normalized response) through GraphPad Prism 8.0.1.

$I (\%) = ((A0 - As)/ A0) \times 100$

Ferric Reducing Antioxidant Power

Ferric Reducing Antioxidant Power (FRAP) of E. mollis aqueous extract was evaluated considering its capacity to reduce Fe^{3+} to Fe^{2+} (Benzie and Strain, 1996). One (1) mL of plant extract at different concentrations (0.1-1 mg/mL) was mixed with 2.5 mL of 0.2 M phosphate buffer solution (pH 6.6) and 2.5 mL of 1% potassium ferricyanide $K_3Fe(CN)_6$ solution. The mixture was incubated in a water bath at 50 °C for 20 min, then 2.5 mL of 10% trichloro-acetate (TCA) solution was added to the mixture to stop the reaction and the tubes were centrifuged at 3000 rpm for 10 min. An aliquot (2.5 mL) of supernatant was combined with 2.5 mL of distilled water and 0.5 mL of 0.1% aqueous iron chloride ($FeCl_3$) solution. The absorbance of the reaction medium was read at 700 nm wavelength against a similarly prepared blank, replacing the extract with distilled water which allows calibration of the apparatus (UV-VIS spectrophotometer). An increase in absorbance corresponds to an increase in the reducing power of the extract of interest tested. Results were expressed as µmol Fe(II)/g of dry extract. The EC_{50} values (the effective concentration providing 0.5 of absorbance or 50% of maximal antioxidant capacity) were calculated from the dose-response curves using linear or nonlinear regression analysis.

Total Antioxidant Capacity

Total Antioxidant Capacity (TAC) of E. mollis aqueous extract was determined using the phosphomolybdenum method described by Prieto et al. (1999). Zero-point two (0.2) mL of plant extract of varying concentrations (0.1-1 mg/mL) was mixed with 2 mL of working reagent solution (0.6 M H_2SO_4 98%, 28 mM NaH_2PO_4 and 4 mM ammonium molybdate). The tubes were

nitric screwed down, and incubated at 95°C for 90 min. After cooling, the absorbance of the solutions was measured at the wavelength of 765 nm against the reagent blank (2 mL of the reagent solution + 0.2 mL of distilled water) treated under the same conditions as the samples. Ascorbic acid (100 - 100 µg/mL) was used as the standard. Results expressed as milligrams of ascorbic acid equivalents per gram of dry extract (mg AAE/g). The EC50 values (the effective concentration providing 0.5 of absorbance or 50% of maximal antioxidant capacity) were calculated from the dose-response curves using linear or nonlinear regression analysis.

Animals

Male albino Wistar rats (6-8 weeks old) were used for the experiment. They were raised 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), Yaounde, Cameroon, under natural conditions of light and temperature, with free access to water and regular roden chow. In vivo experiments were conducted according to the Guidelines of the European Union directive on Ethical Evaluation of Animal Experiments (CEE Council 86/609) (Smith et al., 2007) and ethically approved by the Institutional Committee of the Ministry of Scientific Research and Innovation of Cameroon.

Experimental Design

The rats selected for the study were randomly distributed into six group of six animals each (n=6):

- One group of Normal Control (NC) received distilled water and normal diet (Kamgang et al., 2005);
- One group of High Fat Diet Control (HFDC) received distilled water and high fat diet (Kamgang et al., 2005);
- One group of positive Control (Met) received the reference drug, Metformin (70.0 mg/kg) and high fat diet;
- Three groups received high fat diet and respectively 50 (Em50), 100 (Em100) or 200 (Em200) mg/kg b.w. E. mollis aqueous extract.

The animals were treated once daily by oral gavage at a volume of 1 mL/100 g body weight for 30 consecutive days. During the experimental period, Food and water intake were recorded every two days, body weight was monitored every five days, and fasting glycemia was measured at the beginning, then every ten days. At the end of treatment, the oral glucose tolerance test (OGTT) as well as the insulin tolerance test (ITT) were performed after 12 hours of fasting. Following these tests, animals were sacrificed under anesthesia (diazepam, 10 mg/kg and ketamine, 50 mg/kg). Visceral, perirenal and peritesticular white adipose tissues were recollected and weighted, carcasses were taken and weighed.

Oral Glucose Tolerance Test

The Oral Glucose Tolerance Test (OGTT) was performed at the end of treatment according to Ngakou et al (2023). After a 12 hrs. fasting period, rats received an oral glucose load (2.5 g/kg body weight D-glucose, Central Drug House). Blood glucose level was measured just before administration and at 30, 60, and 120 min after oral glucose administration. The area under the curve (AUC) was calculated using the trapezoidal method.

Insulin Tolerance Test

The Peripheral insulin sensitivity (ITT) was evaluated as described by Mvongo and collaborators (2014) with slight modifications. Following a 12 h fasting period, blood glucose was recorded before subcutaneous administration of insulin (Actrapid Human HM, 2 UI/kg b.w.). Then blood glucose level was measured at 15, 30, and 60 min after insulin administration.

Statistical Analysis

The results are expressed as mean ± standard error of mean (SEM) and statistically analyzed by one-way analysis of variance (ANOVA) associated with a Turkey test followed by Dunnett test, using Graph pad Prism 8.0.1. The difference between and within various groups was significant with $p< 0.05$.

3. RESULTS

Phytochemical Profile

Phytochemical screening

Phytochemical screening of E. mollis (Em) extract revealed the presence of bioactive secondary metabolites, such as phenols, tannins, anthraquinones, saponins, sterols, flavonoids, anthocyanidins, coumarins, triterpenes, alkaloids, and polysaccharides.

Total polyphenols, flavonoids, tannins, alkaloids and saponins content

Following the quantitative phytochemical analysis of E. mollis aqueous extract, saponins were the most abundant quantified metabolites (907.5 ± 18.37 µg SE/g), followed respectively by total polyphenols (788.67 ± 11.58 µg GAE/g), flavonoids (189,02 ± 7,96 µg QE/g), alkaloids (108,52 ± 9,75 µg QiE/g) and tannins (5,25 ± 0,18 µg TAE/g) (Tab. 1).

Table 1: Total polyphenols, flavonoids, tannins, alkaloids and saponins content of E. mollis aqueous extract

	Total polyphenols (µg GAE /g DM)	Flavonoids (µg QE /g DM)	Tannins (µg TAE /g DM)	Alkaloids (µg QiE /g DM)	Saponins (µg SE/g DM)
Quantity	788,67 ± 11,58	189,02 ± 7,96	5,25 ± 0,18	108,52 ± 9,75	907,5 ± 18,37

µg: Micrograms; **GAE:** Gallic Acid Equivalent; **QE:** Quercetin Equivalent; **TAE**: Tannic Acid Equivalent; **EQi:** Quinine Equivalent; **SE:** Saponins Equivalent; **g:** Grams; **DM:** Dry Matter; n=3

In Vitro Antioxidant Activity

DPPH free radical scavenging and Nitric oxide radical inhibiting activities:

In concentration dependent manner, the scavenging effect of E. mollis aqueous extract on DPPH (1.1- Diphenyl-2-picrylhydrazyl) free radical increased (Fig 1A) with an IC_{50} value of 1.38 ± 0.04 mg/mL (Tab 2).

The inhibiting activity of E. mollis aqueous extract on the nitric oxide (NO) radical increased in concentration dependent manner (Fig 1B), with an IC_{50} of > 4.00 mg/mL (Tab 1).

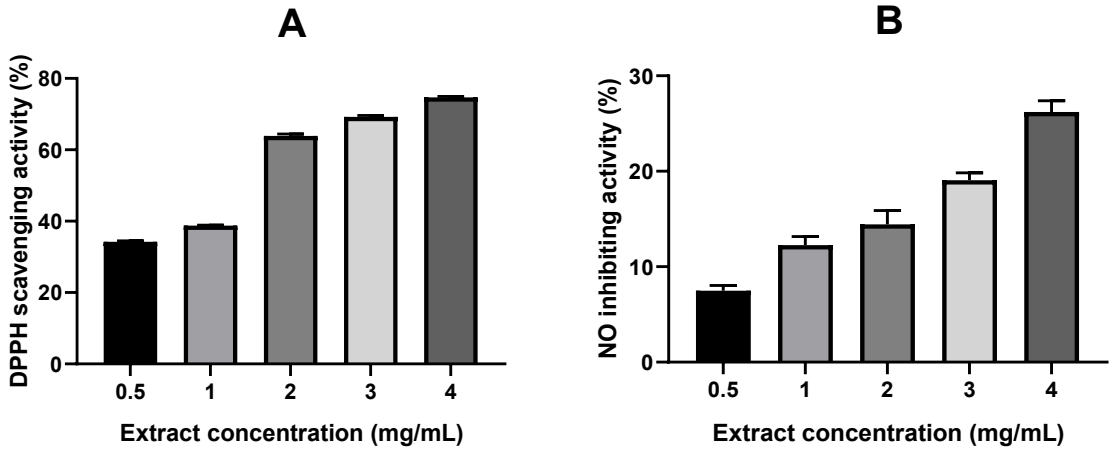


Figure 1: DPPH scavenging (A) and nitric oxide inhibiting (B) activity of E. mollis aqueous extract. DPPH:1.1- Diphenyl-2-**picrylhydrazyl. Significant difference. n=3**

Ferric Reducing Antioxidant Power and Total Antioxidant Capacity

Ferric reducing antioxidant power (FRAP) of E. mollis aqueous extract increased progressively dose dependently (Fig 2A) with an EC_{50} value of 0.48 ± 0.02 mg/mL (Tab 2).

Total antioxidant capacity (TAC) of E. mollis aqueous extract, assessed by the phosphomolybdenum method, remained consistently high for the concentrations, ranging from 449.156 to 530.267 mg equivalent of ascorbic acid /g of extract (Fig 2B), with an EC_{50} of < 0.10 mg/mL (Table 2).

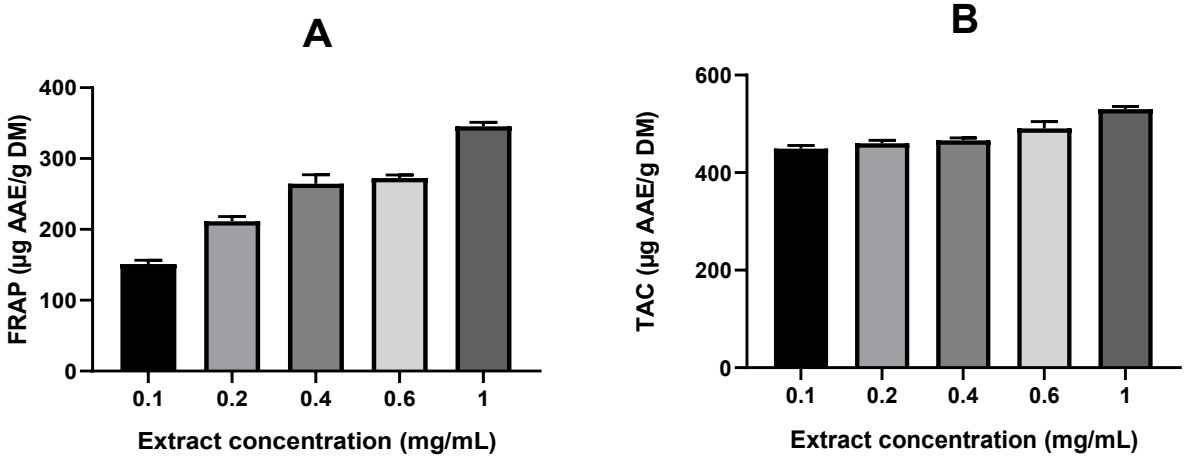


Figure 2: Ferric Reducing Power (A) and Total Antioxidant Capacity (B) of E. mollis aqueous extract. FRAP: Ferric Reducing Antioxidant Power; TAC: Total Antioxidant Capacity; AA: Ascorbic Acid, n = 3.

The IC_{50} and EC_{50} values summarize the antioxidant potential of the extract. The IC_{50} for DPPH was 1.38 ± 0.04 mg/mL, while it exceeded 4.00 mg/mL for oxide inhibition. Regarding reducing power, the EC_{50} for FRAP was 0.58 ± 0.02 mg/mL, and the TAC exhibited the highest potency with an EC_{50} of less than 0.10 mg/mL (Table 2).

Table 2: IC_{50} and EC_{50} Values of E. mollis aqueous extract

	DPPH (mg/mL)	NO inhibition (mg/mL)	FRAP (mg/L)	TAC (mg/mL)
IC_{50}	1.38 ± 0.04	> 4.00 mg/mL	-	-

EC_{50}	-	-	0.58 ± 0.02	< 0.10

DPPH:1.1- Diphenyl-2-picrylhydrazyl; NO: Nitric Oxide; FRAP: Ferric Reducing Power; TAC: Total Antioxidant Capacity; $IC_{50:}$ concentration required to inhibit 50% of radicals; $IC_{50:}$ effective concentration providing 0.5 of absorbance or 50% of maximal antioxidant capacity.

Effect of E. mollis aqueous extract on Body Weight gain and food intake

During the treatment, the body mass gain of experimental animals gradually increased when compare to initial value. This body weight gains of high fat diet (HFD) fed rats remained lower compare to normal control (NC). However, 200 mg/kg b.w. E. mollis extract and metformin, respectively from the 20th and 15th day of treatment, slowed down the body mass gain compared to HFD control rats (Fig 3A).

Food intake of HFDC significantly ($p< 0.01$) increased from the 12th day of treatment when compared to initial value. Plant extract remarkably ($p< 0.01$) reduced food intake from the12th day at the doses of 50 and 100 mg/kg b.w. and from the 18th day with 200 mg/kg b.w. when compared to HFDC. The most pronounced and sustained decrease of food intake was observed with 100 mg/kg b.w. E. mollis aqueous extract. Metformin on the other hand significantly ($p< 0.01$) decreased food intake only from the18th day of treatment compared to HFDC (Fig 3B).

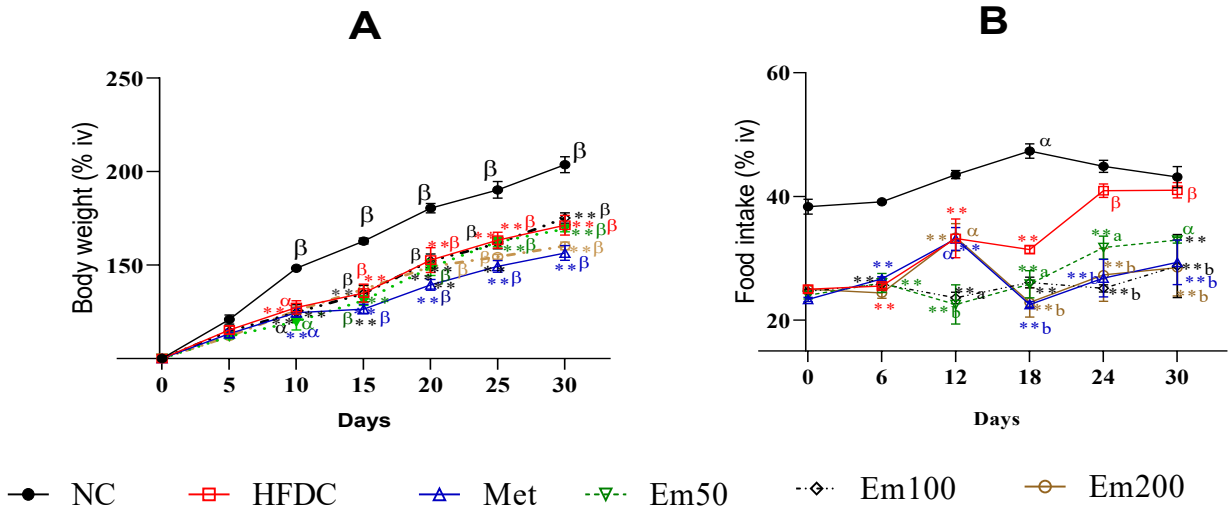


Figure 3: (A) Body weight gain and (B) Food intake (expressed as % of initially values) of rats during 30 days of treatment. NC: normal control rats; HFDC: high fat diet control rats; high fat diet fed rats treated with: 50 mg/kg (Em50), 100 mg/kg (Em100), 200 mg/kg (Em) b.w. E. mollis extract, Metformin 70 mg/kg b.w. (Met). Significant difference: *p< 0.05; **p< 0.01 compared to NC, $^{a}p < 0.05$; $^{b}p< 0.01$ compared to compared to HFDC and $^{\alpha}p < 0.05$; $^{\beta}p< 0.01$ compared to initial value. n=6.

Effect of E. mollis aqueous extract on fasting glycemia and water intake.

During the treatment, MACAPOS 2 high fat diet gradually increased fasting glycemia when compared to normal control rats (NC). On day 10, aqueous extract at the doses used decreased fasting glycemia (- 4.82%, - 5.88 %, - 4.91 % respectively for 50, 100 and 200 mg/kg b.w. plant extract) compared to HFDC, unlike metformin. Subsequently, Elephantopus mollis aqueous extract at a dose of 100 mg/kg b.w. maintained the decrease of fasting glycemia till the end of treatment more than metformin, compared to HFDC (Fig 4A).

High fat diet induced a significant ($p< 0.05$) increased of water intake from day 12 (+10.42%) compared to normal control (NC). Plant extract at a dose of 100 mg/kg b.w. decreased water intake on days 12, 24, and 30 of treatment (-90%, -97.33%, and -93.94%, respectively) compared to HFDC (Fig 4 B).

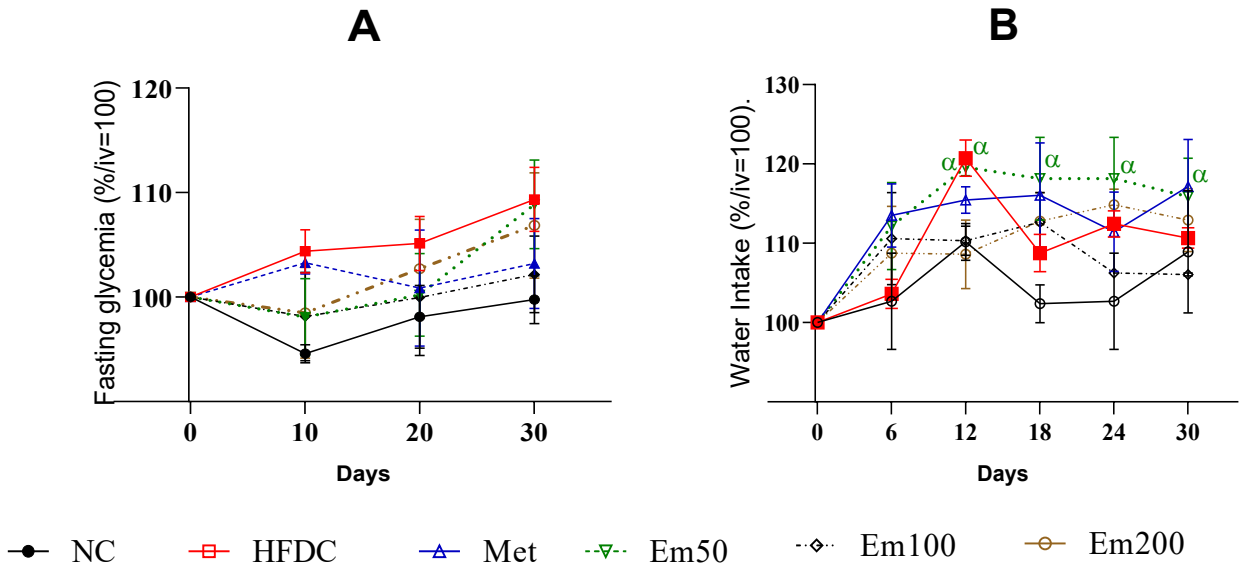


Figure 4: (A) Fasting glycemia and (B) water intake (expressed as % of initially values) of rats during 30 days of treatment. NC: normal control rats; HFDC: high fat diet control rats; high fat diet fed rats treated with: 50 mg/kg (Em50), 100 mg/kg (Em100), 200 mg/kg (Em) b.w. E. mollis extract, Metformin 70 mg/kg b.w. (Met). Significant difference: *p< 0.05 compared to NC, [a]p < 0.05 compared to HFDC and [α]p < 0.05 compared to initial value. n=6.

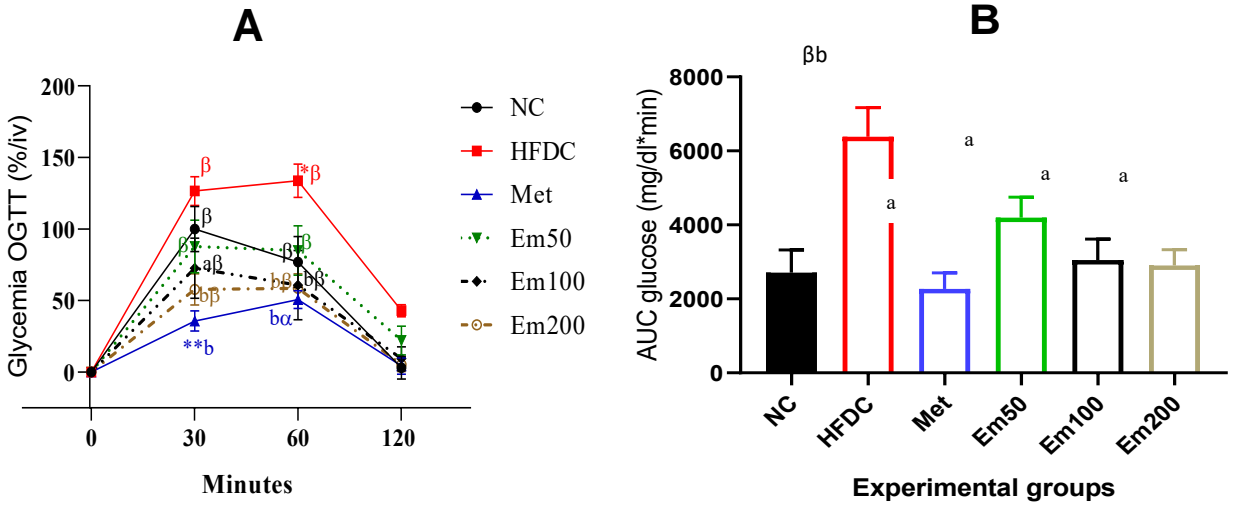
Effect of E. mollis aqueous extract on glucose tolerance and peripheral insulin sensitivity

Glucose Tolerance

Following oral glucose loading, glycemia of high fat diet control rats (HFDC) increased at 30 and 60 min. Metformin and plant extract, at 100 and 200 mg/kg b.w. significantly inhibited the increase of glycemia from the 30th min to 120th min, compared to HFDC. At 60 minutes, glycaemia was reduced by 16.20% (100 mg/kg bw) and 63% (200 mg/kg bw) compared to HFDC (Fig 5A). Area under the curve (AUC) analysis confirmed that plan extract significantly improved glucose tolerance and reduced diet-induced glucose intolerance, with a reducing about 50% for AUC in Em100 and Em200 compared to HFDC (Fig 5B).

Peripheral insulin sensitivity

During the insulin sensitivity assessment, the glycemia of all animals considerably (p <0.01) decreased from the 15th minute after insulin administration, compare to initial value. Between the 30th and 60th minute, glycemia of high fat diet fed control rats (HFDC) remained stable and higher than normal control (NC). Furthermore, plant extract at the dose of 200 mg/kg b.w. resulted in a more rapid decrease of glycemia than that observed in the NC during the 60 minutes following insulin administration (Fig 5C).



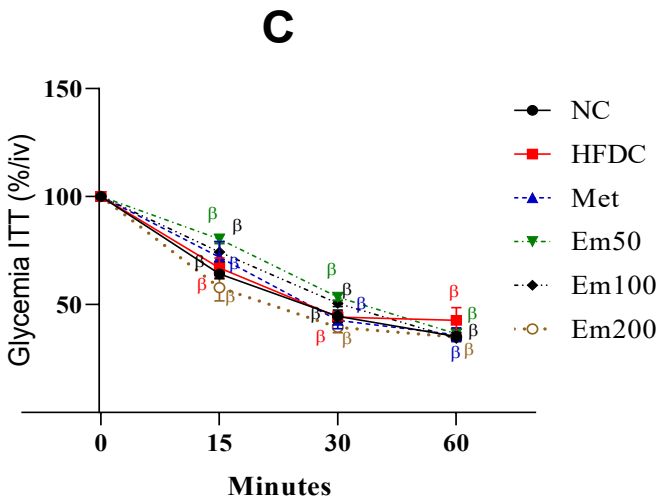


Figure 5: Glycemia variation (expressed as % of initial values) in rat during the OGTT (A), the insulin tolerance test (C) and area under the curve of OGTT (B), after 30 days of treatment. NC: normal control rats; HFDC: high fat diet control rats; high fat diet fed rats treated with: 50 mg/kg (Em50), 100 mg/kg (Em100), 200 mg/kg (Em) b.w. E. mollis extract, Metformin 70 mg/kg b.w. (Met). Significant difference: *p< 0.05; **p< 0.01 compared to NC, ^{a}p < 0.05; ^{b}p< 0.01 compared to HFDC and $^{\alpha}$p < 0.05; $^{\beta}$p< 0.01 compared to initial value. n=6.

Effect of E. mollis aqueous extract on white adipose tissues and carcass

After 30 days of treatment, MACAPOS 2 high fat diet induced significant (p< 0.01) accumulation of visceral, perirenal and peritesticular white adipose tissues, compared to normal control (NC). At doses of 100 and 200 mg/kg b.w. plant extract remarkably decreases (p<0.01) visceral fat: -21.7% and -24.92% respectively compared to HFDC. Metformin, on the other hand, showed no beneficial effect on visceral fat. Plant extract lightly decreased perirenal fat at the dose of 50 et 100 mg/kg b.w. compared to HFDC. better than metformin, E. mollis aqueous extract at the doses used significantly (p<0.01) reduced peritesticular fat (Table 3).

Table 3: **Relative mass of white adipose tissues (Visceral, perirenal and peritesticular fats) and carcass of rats after 30 days of treatment.**

	NC	HFDC	Met	Em50	Em100	Em200
Visceral fat (%)	0.186 ± 0.047	2.163 ± 0.118**	1.675 ± 0.074**	1.619 ± 0.048**	1.468 ± 0.112**a	1.326 ± 0.112**b
Peri-renal fat (%)	0.8430 ± 0.092	2.679 ± 0.301**	2.252 ± 0.326*	2.090 ± 0.291*	1.877 ± 0.322*	2.533 ± 0.130**
Peri-testicular fat (%)	1.125 ± 0.242	2.339 ± 0.524 **	1.887 ± 0.203b	1.551 ± 0.352b	1.316 ± 0.385b	1.505 ± 0.223b
Carcass (%)	72.81 ± 2.63	73.333 ± 3.26	75.949 ± 2.13	72.458 ± 3.73	75.238 ± 3.21	76.419 ± 2.43

NC: normal control rats; HFDC: high fat diet control rats; high fat diet fed rats treated with: 50 mg/kg (Em50), 100 mg/kg (Em100), 200 mg/kg (Em) b.w. E. mollis extract, Metformin 70 mg/kg b.w. (Met). Significant difference: *p< 0.05; **p< 0.01 compared to NC, ^{a}p < 0.05; ^{b}p< 0.01 compared to compared to HFDC. n=6

4. DISCUSSION

The present study was undertaken to assess the phytochemical profile, in vitro antioxidant activity, and the effect of Elephantopus mollis aqueous extract on glycaemia reactivity and anthropometrics parameters on MACAPOS 2 high-fat diet fed rats. The phytochemical screening of E. mollis aqueous extract revealed the presence of several bioactive secondary metabolites such as, phenols, tannins, anthraquinones, saponins, sterols, flavonoids, anthocyanidins, coumarins, triterpenes, alkaloids, and polysaccharides. Quantitative phytochemical analysis indicated that saponins were the most abundant quantified metabolites (907.5 ± 18.37 µg SE/g), followed respectively by phenolic compounds (788.67 ± 11.58 µg GAE/g), flavonoids (189.02 ± 7.96 µg QE/g), alkaloids (108.52 ± 9.75 µg QiE/g) and then tannins (5.25 ± 0.18 µg TAE/g). All those bioactive compounds are known as antioxidants. Polyphenols and flavonoids are widely recognized for their redox-modulating properties, acting as electron or hydrogen donors capable of neutralizing reactive oxygen and nitrogen species (Kasote et al., 2015).

In vitro antioxidant potential of E. mollis aqueous extract was determined hydrogen donating or DPPH (1,1-Diphenyl-2-picrylhydrazyl) free radical scavenging activity, nitric oxide

radical inhibiting activity, ferric reducing antioxidant power (FRAP), and total antioxidant capacity (TAC). The plant extract exhibited important DPPH radical scavenging ability which increased in a dose dependent manner. This result suggests that E. mollis extract is capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. In fact, DPPH is a stable free radical that accepts an electron of hydrogen radical to become a stable diamagnetic molecule. Thus, the increase of DPPH inhibiting percentage (reduction of DPPH absorption) is indicative of the capacity of the extracts to scavenge free radical independently of any enzymatic activity (Li and Li, 2011). This might be due to the total polyphenols, flavonoids and saponins contents of extract which are known as powerful antioxidants. The ferric ions reducing antioxidant power of Em aqueous extract increased with the concentration. This may be a notable indicator of its antioxidant potential. In fact, there is a direct correlation between antioxidant potential and reducing power of an antioxidant (Floegel et al., 2011). The dose-dependent increase in DPPH scavenging and ferric reducing power confirms that the aqueous extract of E. mollis acts as a potent electron donor, a property directly correlated with its high content of polyphenols and flavonoids. The phosphomolybdenum assay indicated a high total antioxidant capacity of plant extract. In a concentration dependent manner, exhibited good nitric oxide inhibiting activity. Nitric oxide inhibition may be benefit to prevent peroxinitrites production, thus prevents damage to biomolecules. The antioxidant potential of E. mollis extract could be due to polyphenols and flavonoids contents which are known as powerful antioxidants. Oxidative stress is a central mechanism linking high-fat diets to insulin resistance, adipocyte hypertrophy, and hepatic lipid accumulation (Roberts & Sindhu, 2009). By mitigating reactive species formation E. mollis aqueous extract may preserve insulin signaling pathways and cellular metabolic integrity (Shehadeh et al., 2021).

The MACAPOS 2 high fat diet successfully reproduced key features of diet-induced metabolic dysregulation, including elevated fasting glycaemia, insulin resistance, glucose intolerance and visceral fat expansion (Kamgang et al., 2005). During 30 days of treatment, E. mollis aqueous extract remarkably decrease food intake. This result suggests that, plant extract modulate satiety signals or interfere with nutrient absorption. In addition, Saponins and phenolic compounds are known to influence lipid emulsification and intestinal absorption, which may reduce effective caloric uptake (Mesa et Rifqi, 2021). The slowdown in food intake was associated with the slowdown in body weight gain observed in these same animals. This decrease in body weight gain could be linked, on the one hand, to the decrease in food intake and, on the other hand, to the remarkable decrease in fat tissues, much more pronounced with the extract doses of 100 and 200 mg/kg b.w. The beneficial effect of the plant extract on tissue fats may be due to the isolated or combined action of chemical compounds such as phenols, flavonoids, saponins, anthocyanins, which are known as lipid-lowering compounds (Dimitry et al., 2022).

From the first week of treatment, E. mollis aqueous extract decreased fasting glycemia with more marked effect at the dose of 100 mg/kg b.w. Interestingly, while the 100 mg/kg dose demonstrated optimal efficacy in maintaining long-term glycemic control, the 200 mg/kg dose was more effective in enhancing peripheral insulin sensitivity, suggesting a dose-specific modulation of different metabolic pathways. This hypoglycemic effect could be due to the isolated or synergistic action of its flavonoids and tannins contents. Tannins and flavonoids are known to promote α-glucosidase inhibitory activity and protect pancreatic beta cells (Mesa et Rifqi, 2021). The decrease of fasting glycemia by E. mollis aqueous extract was associated with the improvement of glucose tolerance. The attenuation of the glycemic peak by plant extract as well as metformin during the oral glucose tolerance test suggests improved early-phase glucose handling. This effect may involve delayed intestinal glucose absorption, modulation of glucose transporter activity, insulin sensitivity activity or enhanced pancreatic responsiveness (Mesa et Rifqi, 2021). The important decrease of hyperglycemia at 100 and 200 mg/kg b.w. suggests an antihyperglycemic activity of the aqueous extract of Elephantopus mollis at these doses.

Furthermore, the insulin tolerance test revealed the improvement of peripheral insulin sensitivity by plant extract, particularly at 200 mg/kg b.w. more than metformin., At the same dose, this extract remarkably reduced visceral fat at the end of treatment. This result suggests that the extract may improve peripheral insulin sensitivity through other pathways than metformin by reducing visceral fat. In fact, visceral adipose tissue is metabolically active and secretes pro-inflammatory cytokines such as TNF-α and IL-6, which impair insulin signaling and promote systemic insulin resistance (Steppan et al., 2001; Zheng et al., 2024). The significant reduction in visceral fat, particularly at higher dose, likely lowered the systemic release of pro-inflammatory adipokines, thereby mitigating the interference with insulin signaling and restoring glucose uptake in peripheral tissues. Therefore, the reduction in visceral fat mass likely contributed to the improvement in glucose homeostasis observed in treated animals (Hu et al., 2021). In addition, this finding suggests that the extract may preserve or restore insulin signaling pathways, through antioxidant-mediated protection of insulin receptor substrates and downstream signaling components (Cheng et al. (2023). The improvement in glucose homeostasis was associated to the decrease of water intake (Hyun-Jong et al., 2012).

5. CONCLUSION

This study indicated on one hand that, Elephantopus mollis aqueous extract exhibited a significant in vitro antioxidant activity. On the other hand, this extract at the doses used, reduced fasting blood glucose level, improved glucose tolerance, insulin sensitivity and anthropometrics parameters in MACAPOS 2 high-fat diet fed rats. These findings might justify the use of this plant in folk medicine for the treatment of diabetes and obesity conditions.

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