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# Impact of *Ficus vallis-choudae* Leaves on Digestive Enzyme Activity and Cardiovascular Markers in Insulin-Resistance Rats

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## ABSTRACT

**Introduction:** *Ficus vallis-choudae* has been traditionally used in Cameroon to manage disorders related to carbohydrate metabolism. This study aimed to evaluate the insulin-sensitizing effects of its aqueous extract of (AEFVC) in vivo and its inhibitory effects on alpha-amylase and alpha-glucosidase in vitro.

**Methods:** Phytochemical profiling of AEFVC was performed to identify its constituent compounds. Enzymatic assays were conducted to assess alpha-amylase and alpha-glucosidase inhibition. Antioxidant activities, including DPPH radical scavenging and iron-reducing capacities, were also evaluated. Male Wistar rats with type 2 diabetes, induced by a high-fat diet and streptozotocin (35 mg/kg), were treated with AEFVC at doses of 110, 220, or 440 mg/kg for 28 days. Parameters such as body weight, blood glucose, lipid profile, and oxidative stress markers were analyzed.

**Results:** Phytochemical analysis revealed that AEFVC contains diverse compounds, including total phenolics, tannins, saponins, and flavonoids in varying concentrations. The extract showed dose-dependent inhibition of alpha-amylase and alpha-glucosidase, with significant reduction in postprandial glucose levels in sucrose and starch tolerance tests at 440 mg/kg. AEFVC exhibited potent antioxidant activity, evidenced by its DPPH radical scavenging and iron-reducing properties ( $p < 0.05$ ). Additionally, the extract significantly improved serum lipid profiles, reducing total cholesterol, triglycerides, LDL cholesterol, and malondialdehyde while increasing HDL cholesterol, glutathione, and catalase levels ( $p < 0.05$ ).

**Conclusion:** AEFVC demonstrated hypoglycemic, antioxidant, and insulin-sensitizing effects, likely mediated through the inhibition of alpha-glucosidase and alpha-amylase. These findings suggest that AEFVC may have therapeutic potential in managing type 2 diabetes and associated metabolic disorders.

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## Introduction

Elevated blood glucose levels exceeding 126 mg/dL or postprandial blood sugar levels above 200 mg/dL are indicative of diabetes mellitus, a dangerous and chronic metabolic condition. Type 1 diabetes is brought on by a relative or total lack of insulin, while type 2 diabetes is brought on by insulin resistance linked to a malfunction in insulin secretion. The autoimmune reaction that causes type 1 diabetes (T1D) occurs when the body's immune system destroys the  $\beta$  cells in the pancreas, which are in charge of making insulin. Type 2 diabetes (T2D) represents a heterogeneous form with a multitude of underlying physiopathological mechanisms. It commences with a gradual decline in insulin efficacy (insulin resistance), subsequently leading to an irreversible deterioration in  $\beta$ -cell functionality and, consequently, insulin secretion (insulin deficiency) (WHO, 2021). Currently, over 420 million individuals worldwide are affected by diabetes (IFD, 2019). In Africa, the number of individuals with diabetes is projected to increase to 28.6 million by 2030 and 47.1 million by 2045 (WHO, 2021). In diabetic patients, one effective method for combating postprandial hyperglycaemia is to focus on the inhibition of digestive enzymes (Cheng and Funtus, 2005). Despite the availability of modern treatments, the prevalence and incidence rate of diabetes continue to rise worldwide, representing a significant public health challenge. Oxidative stress has been identified as a potential contributing factor to the development of diabetes, as well as a potential trigger for associated complications. Antioxidants are naturally present in our diet, but the quantities consumed are often insufficient to meet the body's needs. The use of synthetic antioxidant molecules is currently being questioned due to the potential toxicological risks associated with them. As a result, natural plant extracts are being explored as alternative therapeutic agents. *Ficus vallis-choudae* Delile is a member of the Moraceae family of plants. It is a shrub or small tree, reaching a height of approximately 8 metres. The leaves are simple, alternate and leathery. They are oval in shape, measuring up to 20 x 22 cm, with a smooth upper surface. The stipules are deciduous and range in length from 1 to 3 cm, while the petiole can reach up to 8 cm. Solitary figs, axillary leaves, and a globose shape with a slight flattening at the top are characteristics of the plant. According to Orban et al. (2006), the fruits are solitary figs that are found on the leaf axils. A local treatment for ailments like diabetes, jaundice, nausea, bronchial, and gastrointestinal issues is a decoction of the leaves and young leafy stems of *Ficus vallis-choudae* (Orban et al. 2006). Children eat this plant's figs and think highly of them (Vivien and Faure, 1996).

*Ficus vallis-choudae* Delile has been shown in earlier research to possess a variety of biological activities, such as antifungal, anticonvulsant, anti-inflammatory, and antinociceptive qualities (Adekunle et al., 2005;

Lawan et al., 2008). This study aimed to determine the insulin-sensitizing effects of AEFVC *in vivo* as well as its inhibitory and antioxidant capabilities on the enzyme's alpha amylase and alpha glucosidase *in vitro*.

## Materials and Methods

### Chemicals and Drugs

The following reagents were procured from Sigma-Aldrich (St. Louis, USA): Streptozotocin, Acarbose, alpha-glucosidase, alpha-amylase, 3,5-dinitrosalicylic acid, p-nitrophenyl-D-glucoside (pNPG) and DPPH, Ferric reducing antioxidant potential. The starch and sucrose were procured from the Edu-Lab Biology Kit (Bexwell, UK). All other chemicals and drugs utilized were of analytical grade and commercially available.

### Animal

Male wistar strain rats weighing between 220 and 250g were the study's subjects. The rats were between two and three months old. These animals were kept in the University of Maroua's Physiology and Pharmacognosy Laboratory, where they had unrestricted access to food and water. Animal were housed in polypropylene case under standard environmental conditions (temperature  $22\pm 2^\circ\text{C}$  in a light/dark cycle of 12h). They had fourteen days to become acclimated before the manipulation began. All animal experiments were handled according to the Cameroon National Ethics Committee (Ref. No. FWIRB 00001954), and all experiments were examined and approved.

### Plant Material, Preparation of Extract and Determining the Different Doses

The leaves of the *Ficus vallis-choudae* plant were the subject of our investigation. They were gathered in the Koza district of Cameroon, cleaned with tap water, allowed to dry in the sun, and then ground into a fine powder. By comparing it to the specimen documented under number 5115 SRF/Cam, the plant was identified at the National Herbarium of Yaoundé, Cameroon. To make the AEFVC, we weighed 19.84g of the resulting powder and macerated it for 48 hours in 0.5L of distilled water. Whatman No. 1 paper was then used to filter the mixture. 2.55 g of crude extract mass was obtained by evaporating the filtrate in an oven set to  $45^\circ\text{C}$  for 72 hours. The extraction yield was 12.85%.

$$\text{Extraction yield(\%)} = \frac{\text{Gross Weigh of extract}}{\text{sample weight}} \times 100$$

Adult patients receive 2550 mg of *Ficus vallis-choudae* daily from the traditional healer. The amount of extract ingested by a 70 kg adult allowed for the determination of the 35.71 mg/kg human therapeutic dose. Rats received an analogous dose of around 220 mg/kg, which was determined using Reagan-Shaw et al.'s formula (Reagan-Shaw et al., 2011). For this test, the doses that were used were 110, 220, and 440 mg/kg.

## Phytochemical Screening

### Quantification of Total Phenol

The total phenol content was determined using the Folin–Ciocalteu reagent (Vermerris and Nicholson, 2006). A solution comprising 4 mL of 7.5% w/v sodium carbonate, 2.5 mL of the 10% Folin–Ciocalteu reagent, and 0.5 mL of standard gallic acid or AEFVC was prepared and left to stand at room temperature for 30 minutes. The absorbance of the combination was measured at 727 nm. The total phenol content was expressed in milligrams of gallic acid equivalents per gram (mg GAE/g) of extract.

### Quantification of Total Flavonoid

To ascertain the total flavonoid content, the Miliuskas protocol (Miliuskas et al., 2004) was adhered to. One milliliter of AEFVC or quercetin (standard), 0.2 milliliters of aluminum chloride (10% w/v), 0.2 milliliters of potassium acetate (1 M), and 5.6 milliliters of distilled water were incubated for 30 minutes. The absorbance of the combination was then measured at a wavelength of 415 nm. The milligrams of quercetin equivalents per gram (mg QE/g) were used to quantify the extract's total flavonoid content.

### Quantification of Total Tannin

The total tannin content was calculated using the approach of Bainbridge et al. (1996). 50 µL of standard or AEFVC catechin, 750 µL of a 12 M chloride acid solution, and 1.5 mL of 4% methanol were combined, and the mixture was then allowed to sit at room temperature for 20 minutes. The absorbance of the combination was measured at 500 nm. The total tannin content was expressed in milligrams of catechin equivalent per gram (mg CE/g) of extract.

### Quantification of Total Saponin

The method described by Makkar et al. (2007) was used to determine the total saponin content. 50 µL of AEFVC or diosgenin (standard), 250 µL of distilled water, 250 µL of vanillin reagent (4%), and 2.5 mL of sulfuric acid (72%), were placed in a water bath at 60°C for 10 minutes. The components were all well combined. The absorbance of the mixture was measured at 544 nm after it had been allowed to cool in ice-cold water. The total saponin content was expressed in milligrams of diosgenin equivalents per gram (mg ED/g) of extract.

## In Vitro Enzymes Inhibition Study of AEFVC

### In vitro α-amylase inhibition study

Using Apostolidis et al. (2007)'s calorimetric assay method, which measures the amount of glucose released into the reaction medium, the AEFVC α-amylase inhibition assay was carried out in vitro. 250 µL of AEFVC and 250 µL of 0.02M sodium phosphate buffer (pH=6.9) containing 240 U/mL of the enzyme α-amylase were incubated for 20 minutes at 37°C. The reaction mixture was then

supplemented with 250 µL of 1% starch solution in 0.02 M sodium phosphate buffer. For fifteen minutes, the reaction mixture was incubated at 37°C. After adding 1 mL of DNS to the reaction mixture, it was diluted with 2 mL of distilled water and incubated for 10 minutes in a boiling water bath. The spectrophotometer was used to measure the absorbance at 540 nm. As a positive control, acarbose was employed. Using the formula  $I\% = (Ac - As)/Ac \times 100$ , where Ac is the absorbance of the control and As is the absorbance of the sample, the results were reported as percentage inhibition. Distilled water, which indicates 100% enzymatic activity, served as the control. The regression curve was used to calculate the extract concentration required to 50% inhibit the enzyme's activity (IC<sub>50</sub>).

### α-Glucosidase Inhibition Investigation In Vitro

The α-glucosidase inhibition test was conducted in vitro using a colorimetric assay based on Kim et al. (2006)'s following reaction principle. For 10 minutes, a solution of α-glucosidase enzyme (0.1 U/mL) was added to 150 µL of EAFVC and 100 µL of 0.1 M sodium phosphate buffer (pH = 6.7). The combination was then incubated at 37°C. 200 µL of a 1 mM pNPG solution was added to 0.1 M sodium phosphate buffer following pre-incubation. For 30 minutes, the reaction mixtures were incubated at 37°C. Following incubation, 1 mL of 0.1M Na<sub>2</sub>CO<sub>3</sub> was added, and the spectrophotometer was used to measure the absorbance at 405 nm. IC<sub>50</sub> values were calculated and the % inhibition of the α-glucosidase inhibitory activity was expressed. As a positive control, acarbose was employed.

### Tests for Oral Starch and Sucrose Tolerance

After a 16-hour fast, twenty (25) rats were split into five groups of five rats each at random (Wu et al., 2011). Within ten (10) minutes of the various treatments (extracts at doses 110, 220, 440 mg/kg; acarbose at dose of 10 mg/kg; distilled water at dose 10 mL/kg) being administered, the rats were given either 4 g/kg b.w. of sucrose or 3 g/kg b.w. of starch orally. Before starting the treatment (0 minute) and 30, 60, 90, and 120 minutes after the induction of hyperglycemia, the blood glucose level was monitored using a one-touch glucometer.

- Batch 1 (control) received at dose 10 mL/kg of distilled water
- Batch 2 (standard) got acarbose solution at dose of 10 mg/kg b.w.
- Batch 3 was given 110 mg/kg b.w of AEFVC
- Batch 4 was administered 220 mg/kg b.w of AEFVC
- Batch 5 was administered 440 mg/kg b.w of AEFVC

## Antioxidant Testing of AEFVC In Vitro

### Ferric Reducing Antioxidant Power

The method outlined by Chaouche et al. (2015) was

used to determine the plant extract's capacity to reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$ . 200 mL of extract at different concentrations (0–800 mg/mL), 500 mL of phosphate buffer (2 mM), and 500 mL of  $\text{K}_3\text{Fe}(\text{CN})_6$  (1%) solution were combined to create a solution. The reaction was stopped by adding 500 mL of 10% trichloroacetic acid after it had been incubated for 20 minutes at 50°C. The mixture was then supplemented with 200 mL of 0.1% (w/v)  $\text{FeCl}_3$  and 1.7 mL of distilled water. At 700 nm, the absorbance was measured in relation to the blank. After comparing the results to those of ascorbic acid, the effective concentration ( $\text{EC}_{50}$ ) was determined using the linear regression curve ( $\text{DO} = f(\text{C})$ ).

### DPPH Antiradical Activity

EAFVC's DPPH (1, 1-diphenyl-1-picrylhydrazyl) antiradical impact was assessed using Sayyed et al. (2011)'s methodology. A DPPH solution in 0.5 mM ethanol was made. A test tube containing 3 mL of extract at varying concentrations (0 to 800 g/mL) was then filled with 2 mL of this solution. At room temperature (25°C), the liquid was thoroughly swirled for five minutes. The extract was substituted with distilled water for the control tube. As a positive control, butylhydroxyanisole (BHA) was employed. at 517 nm, absorbance was measured. The following formula was used to express the extract's antioxidant activity as a percentage of inhibition: Where AC is the control absorbance and AS is the sample absorbance, inhibition (%) is equal to  $(\text{AC} - \text{AS})/\text{AC} \times 100$ . The concentration of the plant extract and the positive control required to inhibit 50% of the enzyme ( $\text{IC}_{50}$ ) was calculated.

### Induction of Insulin-Resistance

T2D causes insulin-resistance. Initially, 30 rats were fed a high-fat diet consisting of fat (58%), carbohydrates (17%) and protein (25%) and 5 rats were fed a normal diet (Zhang *et al.*, 2015). After 30 days, only rats with a body mass index (BMI)  $>0.7 \text{ g/cm}^2$  received an injection of Streptozotocin (diluted in 0.01 mol/L sodium citrate buffer, pH 4.4) at a dose of 35 mg/kg followed by administration of a glucose solution to avoid hypoglycaemic shock. Three days later, rats exhibiting elevated blood glucose levels ( $>126 \text{ mg/dL}$ ) were selected for inclusion in the study.

### Distribution and Treatment of Animals

At the end of the induction of insulin resistance, 30 rats were divided into six groups of 5 rats each: 25 insulin-resistant rats and 5 normal rats. Each group was treated for 4 weeks as follows:

Batch 1(normal control): normal rats + distilled water (10 mL/kg)

Batch 2 (insulin-resistant diabetic control): insulin-resistant rats + distilled water (10 mL/kg)

Batch 3: insulin-resistant rats + metformin (40 mg/kg)

Batch 4: insulin-resistant rats + AEFVC (110 mg/kg)

Batch 5: insulin-resistant rats + AEFVC (220 mg/kg)

Batch 6: insulin-resistant rats + AEFVC (440 mg/kg)

Using an electronic balance, the rats were weighed at the beginning of the experiment and then once a week for a total of 28 days. Using the following formula, the BMI was determined on the first day and at the end of the induction period:  $\text{Body mass (g)}/\text{size}^2 (\text{cm}^2)$  equals BMI. In this case, size was ascertained by using a ruler to measure the rat's distance from its muzzle to its anus.

### Collection of Blood and Organs

On the final day of the treatment period, the animals were fasted for a period of 24 hours. They were then anaesthetised via an intraperitoneal injection of ketamine (50 mg/kg bw) and diazepam (10 mg/kg bw). The abdominal cavity was subsequently opened, and a blood sample was collected in tubes lacking anticoagulant properties. This sample was then subjected to centrifugation at 3000 rpm for a period of 20 minutes at a temperature of 4°C. The supernatant was then taken and stored at -20°C for subsequent biochemical parameter analysis. Following the collection of the blood samples, the organs, including the liver and kidneys, were removed and stored for the determination of antioxidant parameters and the pancreas for histological sections.

### Determination of Insulin-Resistance Indices

According to the Matthews et al. (1985) method, the Homeostasis model assessment of insulin resistance (HOMA-IR) was computed using the following formula:  $\text{HOMA-IR} = \text{insulin } (\mu\text{g/L}) \times \text{glycemia (mg/dL)}/22.4$ . A calculation known as  $\text{HOMA-}\beta = 20 \times \text{insulin } (\mu\text{IU/mL})/\text{FBS (mmol/L)} - 3.5$  was used to determine the homeostatic model assessment of  $\beta$ -cell function. (Ma *et al.*, 2014)

### Biochemical Analysis

Glycaemia was quantified at the outset of the experiment and subsequently on a weekly basis for a period of 28 days, using a one-touch glucometer. Serum insulin levels were determined using an enzyme-linked immunosorbent assay (ELISA) kit specific for rat insulin. Total cholesterol (TC), triglycerides (TG), low-density lipoproteins cholesterol (LDL-c) and very-low-density lipoproteins cholesterol (VLDL-c) were assayed in accordance with the methodology proposed by Kaplan (Kaplan, 1984). The atherogenic index (AI) is calculated using the formula  $\text{AI} = \text{Log (TC / HDL-c)}$ , while the coronary artery risk (CRI) is determined by  $\text{IRC} = \text{TC}/\text{HDL-c}$ . The cardioprotective index (CI) is calculated by  $\text{IC} = \text{LDL-c}/\text{HDL-c}$  (Kang *et al.*, 2012; Barter *et al.*, 2007; Quantanilha *et al.*, 2011).

### Determination of Oxidative Stress Markers

Malondialdehyde (MDA) was quantified using the method described by Yagi (Yagi, 1976). Reduced



glutathione (GSH) was determined through the application of the approach outlined by Weckbercker and Cory (1988). Superoxide dismutase (SOD) was assessed using the protocol established by Misra and Fridovich (1972). Catalase (CAT) was evaluated in accordance with the methodology proposed by Aebi (1984).

### Histological Section

Histological slices of the rat pancreas were prepared using the technique outlined by Gabe (1968). The tissue was first preserved in 10% formalin before being dehydrated in progressively stronger alcohol treatments. The tissue was sliced into tiny pieces (4  $\mu$ m) after being impregnated in toluene and then submerged in melted paraffin. Following hematoxylin-eosin (HE) staining, the tissue sections were placed between slides and coverslips using Baume de Canada, a synthetic glue, and examined at a magnification of 250x using a microscope.

### Statistical analysis

The data were presented as mean  $\pm$  standard derivation and analysed using GraphPad Prism software, version 5.03. Two-way analysis of variance (ANOVA) followed by the Bonferroni post-test was employed to analyse bivariate parameters, including variation in blood glucose, body mass. One-way ANOVA and the Tukey post-test were used to analyse single-variable parameters, such as insulin level, HOMA-IR, lipid profile, and oxidative status. A p-value of less than 0.05 was considered statistically significant.

## Results

### Total Content of Phenols, Flavonoids, Tannins, and Saponin

Table 1 presents the bioactive compounds present in AEFVC. A quantitative analysis has revealed the presence of a number of chemical compounds, including total phenols, saponins, tannins and flavonoids. The total polyphenol content, expressed in milligram equivalent of gallic acid per gram of the extract (mgEAG/g), was found to be approximately  $63.06 \pm 0.12$  mg EAG/g. The flavonoids were expressed in milligrams of quercetin per gram of the extract (mg EQ/g), with a value of  $35.55 \pm 0.17$  mgEQ/g. The tannin content, expressed as milligram equivalent of catechin (mg EC/g), was found to be  $34.80 \pm 0.10$  mgEC/g. The saponin content, expressed as milligram equivalent of diogenin per gram of the extract (mgED/g), was determined to be  $19.60 \pm 0.08$  mgED/g.

### In vitro Alpha-Amylase Inhibition Activity of AEFVC

The results showed that the  $\alpha$ -amylase enzyme was inhibited, indicating a concentration-dependent increase in enzyme concentration (Figure 1). Acarbose showed a significant inhibitory ability, as evidenced by inhibition percentages that ranged from 13.77% to 79.97%. On the other hand, the extract showed inhibitory action ranging from 12.38% to 64.85%, which corresponds to doses of 1 to 200  $\mu$ g/mL. The extract's and acarbose's respective IC<sub>50</sub> values were  $32.71 \pm 0.96$  and  $12.93 \pm 0.52$  (Figure 1 and Table 2).

**Table 1:** A quantitative analysis of the chemical compounds presents in AEFVC

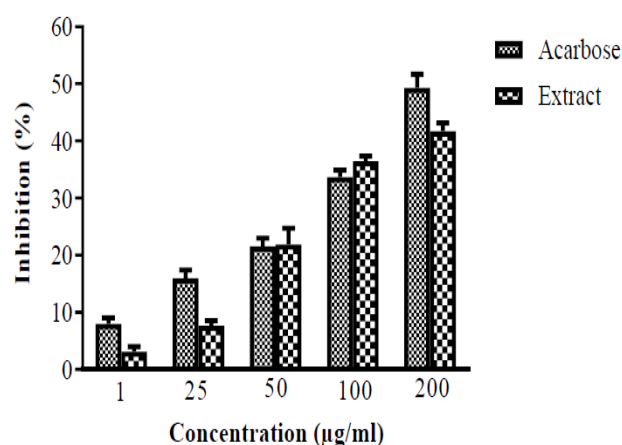
Secondary compounds	Total phenols (mgGAE/g)	Total flavonoids (mgQE/g)	Total tannins (mgCE/g)	Total saponin (mgDE/g)
AEFVC	$63.06 \pm 0.12$	$35.55 \pm 0.17$	$34.80 \pm 0.10$	$19.60 \pm 0.08$

DE (diosgenin equivalent), CE (catechin equivalent), QE (quercetin equivalent), and GAE (gallic acid equivalent); AEFVC: Aqueous extract of *Ficus vallis-choudae*.

**Table 2:** Impact of AEFVC on  $\alpha$ -glucosidase and  $\alpha$ -amylase enzyme decrease

	IC <sub>50</sub> ( $\mu$ g/mL)	
	$\alpha$ - amylase	$\alpha$ - glucosidase
AEFVC	$32.71 \pm 0.96$	$24.08 \pm 1.21$
Acarbose	$12.93 \pm 0.52$	$9.28 \pm 0.66$

IC<sub>50</sub>: The inhibitor's concentration required to block 50% of its action under the test conditions; AEFVC: Aqueous extract of *Ficus vallis-choudae*.

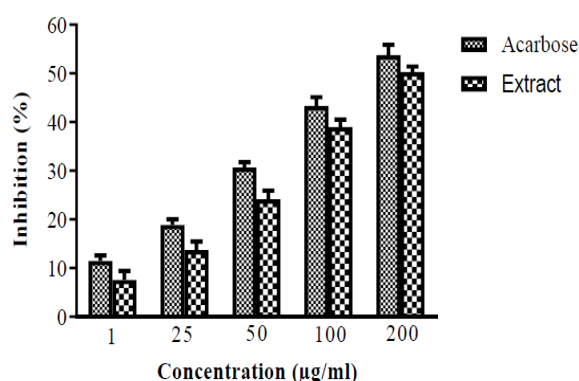


**Figure 1:** AEFVC's ability to inhibit the  $\alpha$ -amylase enzyme.

AEFVC: Aqueous extract of *Ficus vallis-choudae*

### Alpha-Glucosidase Inhibitory Action of AEFVC In Vitro

Figure 2 shows how AEFVC affects the  $\alpha$ -glucosidase enzyme. Analyzing the data showed that AEFVC inhibited the  $\alpha$ -glucosidase enzyme in a manner comparable to that of acarbose, and that this action grew with increasing concentration. Acarbose percentage of inhibition ranged from 19.71 to 63.46% ( $\text{IC}_{50} = 9.28 \pm 0.66 \mu\text{g/mL}$ ), whereas EAFVC percentage of inhibition ranged from 11.89 to 50.71% ( $\text{IC}_{50} = 24.08 \pm 1.21 \mu\text{g/mL}$ ). For doses between 1 and 200  $\mu\text{g/mL}$ , the percentage of inhibition ranged from 11.89 to 50.71% ( $\text{IC}_{50} = 24.08 \pm 1.21 \mu\text{g/mL}$ ).



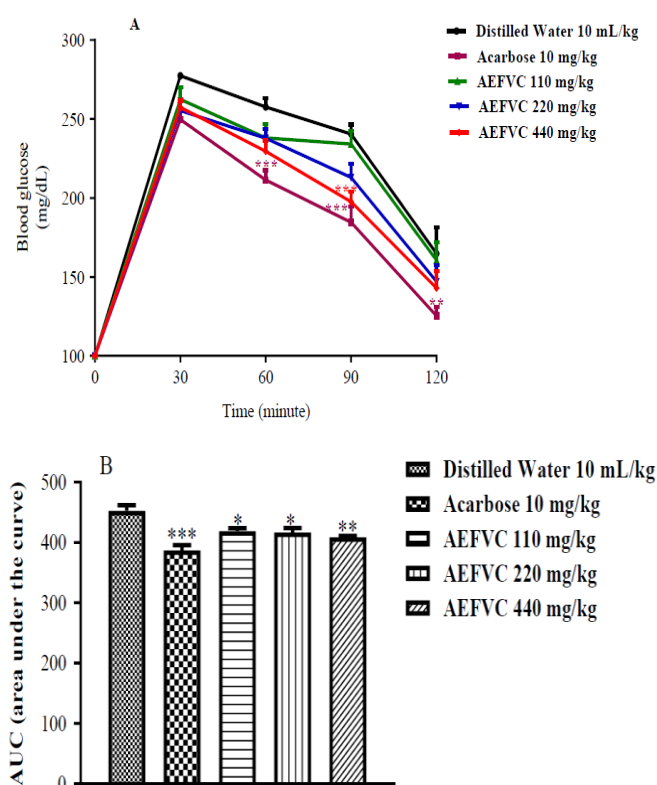
**Figure 2:** EAFVC's ability to inhibit the  $\alpha$ -glucosidases enzyme.

AEFVC: Aqueous extract of *Ficus vallis-choudae*

### Effects of the AEFVC on Oral Starch Tolerance Test

Figure 3 shows the area under the curve (AUC) and the effect of AEFVC on the blood glucose levels of healthy rats participating in an oral starch tolerance test. Following the starch delivery, the blood glucose curves show a spike at the 30-minute mark and a progressive decline until the 120-minute mark. Rats administered the reference product (acarbose) and those administered the AEFVC at a dose of 440 mg/kg showed better control over postprandial

glycemia. At the 60th, 90th, and 120th minutes, respectively, acarbose caused a substantial drop in blood glucose of approximately 42.01% ( $p < 0.001$ ), 47.54% ( $p < 0.001$ ), and 41.96% ( $p < 0.01$ ) in comparison to the control group. Similarly, AEFVC at a dose of 440 mg/kg caused a significant drop in blood glucose at the 90th minute (19, 85%;  $p < 0.001$ ). In contrast to the control group, the acarbose-treated group ( $p < 0.001$ ) and the AEFVC-treated group showed a significant decrease in the area under the curve (AUC) at dosages of 110 and 220 mg/kg ( $p < 0.05$ ) and 440 mg/kg ( $p < 0.01$ ), respectively (Figure 3B).



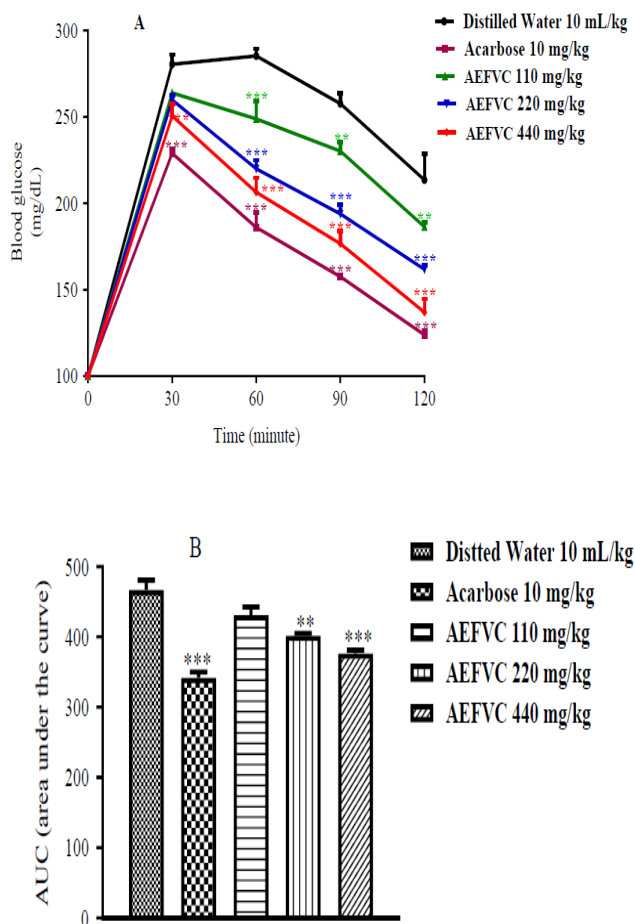
**Figure 3:** AEFVC's impact on the normal rat's oral starch tolerance test (A) and area under the curve following starch loading (B).

AEFVC: Aqueous extract of *Ficus vallis-choudae*; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$  in comparison to control; \* $P < 0.05$ .

### Effects of AEFVC on Oral Sucrose Tolerance Test

Panels A and B of Figure 4 display the results of the sucrose tolerance test. The curves showing blood glucose variations rise at the 30-minute mark and then progressively fall until the 120-minute mark following the administration of sucrose, just like in the starch tolerance test. But over the course of the observation period, the rats treated with acarbose and the various extract dosages had lower blood glucose levels than the control group, which had distilled water. Rats administered the reference product (acarbose) and rats administered the AEFVC at a dose of 440 mg/kg showed better control of postprandial glycaemia.

At the 60th, 90th, and 120th minutes, respectively, acarbose caused a substantial drop in blood glucose of approximately 34.78% ( $p < 0.001$ ), 38.67% ( $p < 0.001$ ), and 42.01% ( $p < 0.001$ ) in comparison to the control group. At 60, 90, and 120 minutes, respectively, the extract significantly reduced blood glucose levels by approximately 27.63% ( $p < 0.001$ ), 31.45% ( $p < 0.001$ ), and 35.85% ( $p < 0.001$ ) at a dose of 440 mg/kg. Blood was less significantly reduced by the extract at doses of 220 and 110 mg/kg than by the extract at dose 440 mg/kg. As a result, blood sugar dropped ( $p < 0.001$ ) by roughly 22.90%, 24.76%, and 24.20% at 60, 90, and 120 minutes, respectively, at a dosage of 220 mg/kg. Similarly, extract at 110 mg/kg demonstrated a substantial decrease at 60 and 120 minutes of 12.76% ( $p < 0.001$ ) and 12.70% ( $p < 0.01$ ), respectively. However, compared to the control group treated with distilled water, acarbose ( $p < 0.001$ ) and EAFVC at doses of 220 ( $p < 0.01$ ) and 440 mg/kg ( $p < 0.001$ ) resulted in a considerable decrease in the area under the curve (AUC) (Figure 4B).



**Figure 4:** EAFVC's impact on the normal rat's oral sucrose tolerance test (A) and area under the curve following sucrose loading (B).

AEFVC: Aqueous extract of *Ficus vallis-choudae*; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$  in comparison to control; \* $P < 0.05$ .

### In Vitro Antioxidant Parameters of AEFVC Ferric Reducing Antioxidant Power (FRAP)

The EC<sub>50</sub> values calculated during the ferric antioxidant reducing power test. Ascorbic acid and the extract demonstrated a concentration-dependent reducing capacity. These results indicated that the extract exhibited a lower capacity to reduce iron than ascorbic acid, with the IC<sub>50</sub> of the extract being  $98.30 \pm 2.21 \mu\text{g/mL}$  and that of ascorbic acid being  $80.88 \pm 1.33 \mu\text{g/mL}$ . The concentration of a material that results in a 50% change in absorbance under the given test conditions is known as the effective concentration (EC<sub>50</sub>).

### DPPH Antiradical Activity

The results of the DPPH antiradical activity of the extract and the positive control substance (ascorbic acid) demonstrated a concentration-dependent increase in the percentage inhibition of DPPH to free radicals. The standard demonstrated a high degree of anti-radical activity, with an IC<sub>50</sub> value of  $87.63 \pm 2.41 \mu\text{g/mL}$ , which was higher than that observed for the extract, with an IC<sub>50</sub> value of  $95.90 \pm 3.30 \mu\text{g/mL}$ .

### Effect of AEFVC on Insulin-Resistant Effect of the Extract on Body Weight, BMI and Abdominal Fat

Table 3 illustrates the impact of the extract on body mass, BMI and abdominal fat. The results of the study demonstrated that the administration of pork fat to the animals for a period of four weeks led to a statistically significant increase ( $p < 0.001$ ) in body mass, BMI, and abdominal fat in comparison to the control group. Therefore, the body mass of the normal control group, which was  $234.07 \pm 2.70 \text{ g}$ , increased to  $378.78 \pm 6.66 \text{ g}$  in the group fed a diet rich in fat, representing a 38.20% increase ( $p < 0.001$ ). The BMI increased by 70% ( $p < 0.001$ ), and abdominal fat by 54.90% ( $p < 0.001$ ).

However, following treatment with AEFVC at doses of 110, 220 and 440 mg/kg, a significant decrease ( $p < 0.001$ ) was observed, respectively, of 29.79%, 29.23% and 33%. A reduction of 0.20% in weight; 65.78%, 64.77%, 70.06% in BMI; and 30.20%, 32.98%, 37.54% in abdominal fat was observed in comparison to the diabetic control.

Similarly, metformin resulted in a notable reduction ( $p < 0.001$ ) of 31.50%, 68.98%, and 38.75% in body mass, BMI, and abdominal fat, respectively, in comparison to the diabetic control group.

**Table 3:** Effect of the AEFVC on body weight, BMI and abdominal fat

Parameters	NC	IDC	Metformin (40 mg/kg)	AEFVC		
				110 mg/kg	220 mg/kg	440 mg/kg
Initial weigh (g)	234.07±2.70	248.78±2.66 <sup>a</sup>	241.47±3.12	240.37±1.53	238.72±2.12	237.25±1.12
Weigh after induction (g)	234.07±2.70	378.78±6.66 <sup>a</sup>	381.47±4.12	370.37±1.53	378.72±5.12	365.25±1.12
Weigh after treatment (g)	251.34±2.85	374.62±8.50 <sup>a</sup>	256.59±2.11 <sup>c</sup>	263.01±3.45 <sup>c</sup>	263.98±4.32 <sup>c</sup>	250.24±2.77 <sup>c</sup>
BMI (g/cm <sup>2</sup> )	0.66±0.11	2.20±0.08 <sup>a</sup>	0.68±0.03 <sup>c</sup>	0.75±0.04 <sup>c</sup>	0.78±0.05 <sup>c</sup>	0.66±0.04 <sup>c</sup>
A.F (g)	4.18±0.09	9.27±0.20 <sup>a</sup>	5.67±0.24 <sup>c</sup>	6.47±0.02 <sup>c</sup>	6.21±0.06 <sup>c</sup>	5.79±0.18 <sup>c</sup>

<sup>a</sup> $p < 0.001$  statistically significant compared to the normal control. <sup>c</sup> $p < 0.001$  statistically significant compared to the IDC. BMI: Body Mass Index, AF: Abdominal Fat, NC: normal control, IDC: insulin-resistant diabetic control, AEFVC: aqueous extract of *Ficus vallis-choudae* leaves.

### Effect of AEFVC on Glycaemia

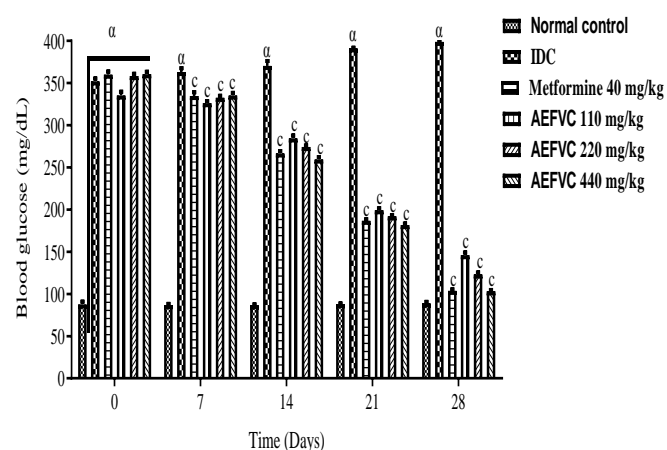
The effects of daily administration of AEFVC at doses of 110, 220 and 440 mg/kg on the blood sugar levels of diabetic rats over a 28-day treatment period following the ingestion of pig fat and a low-dose injection of STZ are presented in Figure 5. A notable elevation ( $p < 0.001$ ) in blood sugar levels was observed in the cohorts fed a diet enriched in pork fat in comparison to the control group, which was maintained on a standard diet. Conversely, the fluctuation in blood sugar levels throughout the 28-day treatment period demonstrates a notable decline in blood sugar levels in comparison to the insulin-resistant diabetic control group. Therefore, on the 28th day of treatment, a significant reduction ( $p < 0.001$ ) of 63.41%, 69.11% and 74.16% was observed with the respective doses of 110, 220 and 440 mg/kg of AEFVC.

The administration of metformin resulted in a statistically significant reduction ( $p < 0.001$ ) of 28.04%, 52.22% and 74.11% on days D<sub>14</sub>, D<sub>21</sub> and D<sub>28</sub>, respectively, in comparison to the insulin-resistant diabetic control group.

### Effect of AEFVC on Insulinemia, HOMA-IR and HOMA-β Indices

Figure 6 summarizes how AEFVC affected insulinemia, HOMA-IR, and HOMA-β after 28 days of therapy. Compared to the normal control, the diabetes control's insulinemia rose by 84.17% ( $p < 0.001$ ). Our study assessed insulin resistance indices including HOMA-IR and HOMA-β. The diabetes control group's HOMA-IR insulin resistance index rose by 87.88% ( $p < 0.001$ ) in comparison to the normal control group. All diabetes controls, however, showed a significant decrease (85.81%;  $p < 0.001$ ) in their HOMA-beta indices as compared to the normal control. But following 28 days of

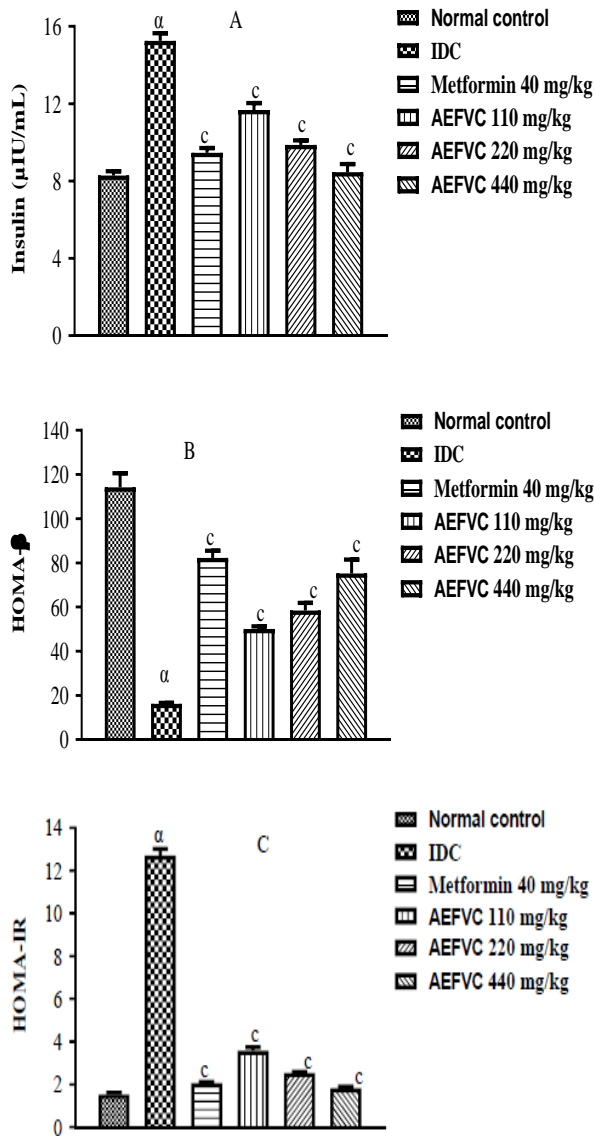
treatment, there was a substantial ( $p < 0.001$ ) drop in insulin concentration, a rise in the HOMA-β index, and a fall in the HOMA-IR index. The percentage of insulin reduction at dosages of 110, 220, and 440 mg/kg of AEFVC in comparison to IDC was 23.47%, 35.27%, and 44.25%, respectively.



**Figure 5:** AEFVC's impact on rats' fluctuating blood glucose levels.

One-way ANOVA and Turkey's post hoc test were used to analyze the data. Substantially significant as compared to the normal control ( $\alpha p < 0.001$ ); Statistically significant as compared to the diabetes control (<sup>a</sup> $p < 0.05$ , <sup>b</sup> $p < 0.01$  <sup>c</sup> $p < 0.001$ ); AEFVC: Aqueous extract of *Ficus vallis-choudae*; IDC stands for insulin resistance diabetic control.



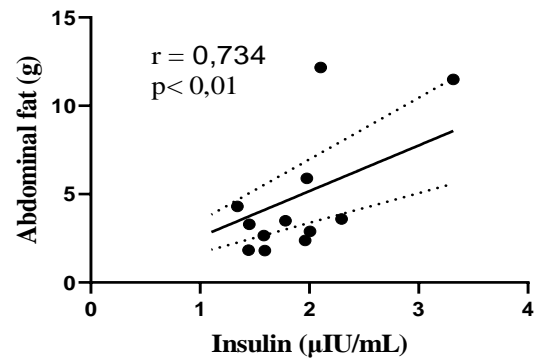


**Figure 6:** AEFVC's impact on HOMA-IR (B), HOMA-β (C), and insulin level (A).

One-way ANOVA and Turkey's post hoc test were used to analyze the data. substantially significant as compared to the normal control group ( $\alpha p < 0.001$ ); statistically significant as compared to the diabetes control group ( $\alpha p < 0.001$ ). AEFVC is an aqueous extract of *Ficus vallis-choudae* leaves; HOMA-IR is an insulin resistance homeostasis model assessment; and HOMA-β is a β-cell function homeostasis model assessment.

### Correlation Between Abdominal Fat and Insulinemia

Figure 7 shows the correlation between variation in abdominal fat and serum insulin level. In type 2 diabetes mellitus, the increase in abdominal fat is accompanied by an increase in insulin levels. However, after 28 days of treatment at different doses of the extract, a reduction in abdominal fat and insulin levels was noted marked by a positive correlation ( $r=0.734$ ;  $p<0.01$ ).



**Figure 7:** Correlation between abdominal fat and insulinemia

### Effect of AEFVC on the Lipid Profile and Cardiovascular Risk Markers

Table 6 demonstrates the influence of the AEFVC on cardiovascular risk markers, including the lipid profile, atherogenic index, cardioprotective index and coronary risk artery of insulin-resistant diabetic rats. The results demonstrated a statistically significant increase ( $p < 0.001$ ) in the serum concentration of total cholesterol in insulin-resistant rats in comparison to the normal control group ( $161.75 \pm 0.98$  mg/dL versus  $95.68 \pm 4.25$  mg/dL). Similarly, a significant increase was observed in triglycerides ( $119.83 \pm 0.70$  mg/dL versus  $90.98 \pm 0.57$  mg/dL), LDL-c ( $126.99 \pm 0.83$  mg/dL versus  $46.30 \pm 4.02$  mg/dL). Furthermore, there was a notable decline in HDL-c levels ( $22.75 \pm 0.45$  mg/dL versus  $47.14 \pm 0.67$  mg/dL).

Conversely, a 28<sup>th</sup> day course of treatment with EAFVC and metformin resulted in a notable reduction in the serum concentration of LDL-c, CT, VLDL-c and TG, accompanied by an increase in the serum concentration of HDL-c, in comparison to the insulin-resistant diabetic control group. Similarly, an increase in the atherogenic index and the coronary risk index, a decrease in the cholesterol index were observed ( $p < 0.001$ ). Consequently, at doses of 110 mg/kg, 220 mg/kg, and 440 mg/kg, our findings demonstrated a significant reduction ( $p < 0.001$ ). The respective reductions in serum CT concentration were 28.57%, 35.12% and 45.22%; in TG, 27.02%, 36.93% and 37.77%; and in LDL-c, 29.76%, 45.58% and 59.38%.

However, AEFVC treatment resulted in a statistically significant increase ( $p < 0.001$ ) of 35.59% and 46.66% at doses of 220 mg/kg and 440 mg/kg, respectively, in serum HDL-c concentration and CI compared to the diabetic control group. The atherogenic index and coronary artery risk were found to decrease significantly in all groups treated with AEFVC. The reference drug metformin, employed in the insulin-resistant diabetic model, resulted in a statistically significant reduction ( $p < 0.001$ ) in the serum concentration of LDL-c (60.25%) and TG (38.83%). Furthermore, there was a notable increase in the concentration of HDL-c (61.53%), as well as a significant rise in the levels of VLDL-c (37.14%) and CT (45.00%) in comparison to the diabetic control group.

**Table 4:** Effect of AEFVC on the lipid profile, atherogenic index, cardioprotective index and coronary artery risk

Parameters (mg/dL)	NC	IDC	Metformin (40 mg/kg)	AEFVC 110 mg/kg	AEFVC 220 mg/kg	AEFVC 440 mg/kg
<b>LDL-c</b>	46.30±4.02	126.99±0.83 <sup>a</sup>	48.66 ±7.05 <sup>c</sup>	89.19±0.70 <sup>c</sup>	69.11±4.07 <sup>c</sup>	51.58±0.58 <sup>c</sup>
<b>TG</b>	90.98±0.57	119.83±0.70 <sup>a</sup>	75.3±1.00 <sup>c</sup>	87.45±0.41 <sup>c</sup>	75.57±1.79	74.57±1.06 <sup>c</sup>
<b>TC</b>	95.68±4.25	161.75±0.98 <sup>a</sup>	88.95±3.85 <sup>c</sup>	115.53±4.10 <sup>c</sup>	104.93±0.68 <sup>c</sup>	88.59±0.60 <sup>c</sup>
<b>VLDL-c</b>	18.19±0.11	23.96±0.14 <sup>a</sup>	15.06±0.20 <sup>c</sup>	17.49±0.08 <sup>c</sup>	15.11±0.35 <sup>c</sup>	14.91±0.21 <sup>c</sup>
<b>HDL-c</b>	47.14±0.67	22.75±0.45 <sup>a</sup>	36.75±0.38 <sup>c</sup>	20.74±0.24 <sup>a</sup>	30.85±0.34 <sup>c</sup>	33.37±0.41 <sup>c</sup>
<b>AI</b>	1.44± 0.22	15.96±0.80 <sup>a</sup>	2.18±0.12 <sup>c</sup>	2.10±0.07 <sup>c</sup>	2.34±0.03 <sup>c</sup>	2.01±0.09 <sup>c</sup>
<b>CI</b>	1.27±0.10	0.12±0.007 <sup>a</sup>	0.87±0.02 <sup>c</sup>	0.71±0.04 <sup>c</sup>	0.74±0.05 <sup>c</sup>	0.79±0.01 <sup>c</sup>
<b>CRI</b>	1.98±0.21	13.35±1.10 <sup>a</sup>	2.19±0.07 <sup>c</sup>	2.47±0.17 <sup>c</sup>	2.41±0.16 <sup>c</sup>	2.28±0.12 <sup>c</sup>

<sup>a</sup>  $p < 0.001$  statistically significant compared with the normal control group. <sup>c</sup>  $p < 0.001$  statistically significant compared with the insulin-resistant diabetic control. NC: normal control, AEFVC: aqueous extract of the leaves of *Ficus vallis-choudae*; CI: cardioprotective index; TC: total cholesterol; TG: triglycerides; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; VLDL-c: very-low-density lipoprotein cholesterol; AI: atherogenic index; CRI: coronary artery risk

### Effect of AEFVC on Oxidative Stress Markers

Table 7 provides a summary of the effects of AEFVC on superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and malondialdehyde (MDA) in the kidney and liver. Following analysis, it was determined that following a high-fat diet and subsequent streptozotocin injection, the concentration of SOD exhibited a notable decline ( $p < 0.001$ ) in the insulin-resistant diabetic group when compared to the normal control. This decline was observed to be 68.28% in the liver and 24.34% in the kidney. The administration of EAFVC to rats resulted in a statistically significant ( $p < 0.001$ ) increase in the activity of this enzyme, with a dose of 440 mg/kg of EAFVC, yielding percentages of 79.99% and 41.05% in the liver and kidney, respectively. A notable decline in catalase activity was observed in the livers and kidneys of the diabetic control group, with a 59.74% and 50.38% reduction, respectively ( $p < 0.001$ ). This was in contrast to the normal control group, where no significant changes were noted. Following treatment with AEFVC, an increase in catalase activity was observed in the liver (163.93%;  $p < 0.001$ ) and kidney (135.64%;  $p < 0.001$ ) with the 220 mg/kg dose, and 212.42% and 138.80% respectively in the liver and kidney with the 440 mg/kg dose.

The percentage reduction in GSH concentration was 61.75% ( $p < 0.001$ ) in the liver and 58.02% ( $p < 0.001$ ) in the kidney. The administration of AEFVC resulted in a notable elevation in glutathione levels within the liver and kidneys of all treated groups, in comparison to the untreated diabetic control group. Nevertheless, the 440 mg/kg extract yielded a notable elevation in this enzyme: 156.31% ( $p < 0.01$ ) and 97.72% ( $p < 0.05$ ), respectively, in the liver and kidney of the 440 mg/kg extract-treated groups.

In contrast to the significant decrease in catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH) activity in the liver and kidney of the diabetic control, there was a significant increase in malondialdehyde (MDA) levels in the various organs studied compared with the normal control. Therefore, there was a significant increase in the concentration of the enzyme in the liver and kidney of the insulin-resistant diabetic control group, with a value of  $94.02 \pm 1.38$  nmol/g ( $p < 0.001$ ), which was 198.71% higher than that of the normal control group. The administration of AEFVC and metformin resulted in a statistically significant reduction ( $p < 0.001$ ) in MDA levels in the liver and kidney when compared with the insulin-resistant diabetic control group.

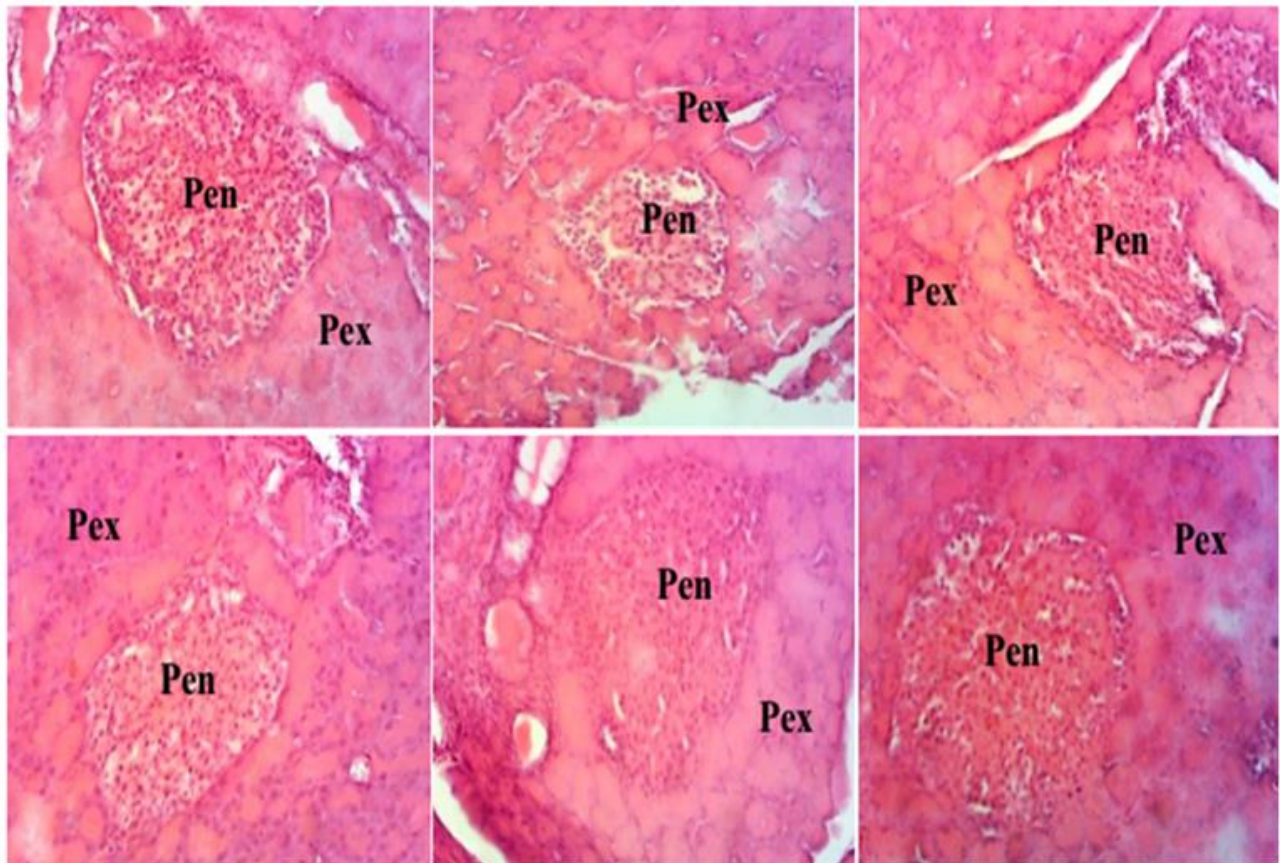
### Effect of AEFVC on Histological Sections of Pancreas

Histological section analysis in insulin-resistant diabetic rats revealed the presence of lobular atrophy and a reduction in the size of the endocrine pancreas in comparison to the control group. In contrast, treatment with AEFVC had a more or less significant impact on the appearance of the islets of Langerhans. In comparison to the IDC (negative control), the histological section of the pancreas of rats treated with 110 mg/kg exhibited a subnormal appearance, with a partial recovery in the size of the islets of Langerhans. Similarly, treatment with 220 mg/kg demonstrated a moderate degree of improvement, accompanied by a moderate increase in islets of Langerhans. Lastly, treatment with a dose of 440 mg/kg resulted in a significant increase in islet size. Following a 28-day treatment period, metformin (positive control), the reference drug used in this study, demonstrated less significant effects on the pancreas than AEFVC (Figure 8).

**Table 5:** effect of AEFVC on serum concentrations of SOD, CAT, GSG and MDA

Parameters	Organs	NC	IDC	Metformin (40mg/kg)	AEFVC		
					110 mg/kg	220 mg/kg	440 mg/kg
<b>SOD</b> (U/mg)	Liver	95.23±1.75	30.2±1.83 $\alpha$	93.83±6.11c	91.78±2.08c	97.19±1.99c	106.14±5.74c
	Kidney	80.91±2.70	61.21±1.37 $\alpha$	72.99±1.40a	74.12±4.30a	80.33±1.4c	86.34±2.41c
<b>CAT</b> ( $\mu$ mol/mg)	Liver	57.40±3.28	23.10±1.27 $\alpha$	59.57±1.49c	55.27±1.53c	60.97±3.05c	72.17±1.26c
	Kidney	59.88±1.91	29.71±1.52 $\alpha$	56.92±1.20c	65.76±2.10c	70.01±4.51c	70.82±0.65c
<b>GSH</b> (nmol/g)	Liver	6.73±0.97	2.57±0.37 $\alpha$	5.31±0.66a	3.76±0.39	5.50±0.36a	6.60±0.34b
	Kidney	5.49±0.54	2.30±0.36 $\alpha$	4.45±0.74	2.38±0.32	4.49±0.50	4.56±0.35a
<b>MDA</b> (nmol/g)	Liver	31.48±1.19	94.02±1.38 $\alpha$	24.19±1.22c	34.01±1.82c	43.53±1.32c	21.39±0.91c
	Kidney	40.21±1.35	69.87±0.56 $\alpha$	31.90±0.92c	41.24±1.75c	30.23±1.57c	27.98±0.88c

NC: normal control, AEFVC: aqueous extract of the leaves of *Ficus vallis-choudae*; SOD: superoxide dismutase; CAT: catalase; GSH: reduced glutathione; MDA: malondialdehyde. NC: normal control; IDC: insulinresistant diabetic control.



**Figure 8:** Effect of AEFVC on histological section of pancreas in insulin-resistant rats (X250; H-E). Pex: exocrine pancreas; Pen: endocrine pancreas; AEFVC is an aqueous extract of *Ficus vallis-choudae* leaves.



## Discussion

Different types of diabetes can now be treated using a variety of therapy techniques. Inhibiting enzymes that break down carbohydrates, such as  $\alpha$ -amylase and  $\alpha$ -glucosidase, is one of the most promising antidiabetic treatment approaches (Narkhede *et al.*, 2011). Complex polysaccharides are hydrolyzed by  $\alpha$ -amylase to create oligosaccharides and disaccharides.  $\alpha$ -glucosidase then hydrolyzes them to make monosaccharides, which are absorbed from the small intestine into the portal vein of the liver (Smith *et al.*, 2005).

$\alpha$ -Amylase and  $\alpha$ -glucosidase inhibitors reduce postprandial glucose levels by delaying the breakdown and subsequent absorption of carbohydrates. By decreasing the pace and magnitude of glucose absorption, the inhibition of these enzymes has a postprandial antihyperglycemic impact (Okoli *et al.*, 2011). Acarbose is one of the common medications used for this. Unfortunately, a number of negative effects and an inability to effectively control exacerbations have been linked to these medicines (Ducobu, 2003). Nevertheless, natural inhibitors of digestive enzymes with fewer adverse effects have been identified in a range of medicinal plants. *F. vallis-choudae* is one of the herbs that has been traditionally employed in the treatment of diabetes. The objective of this study was to assess the inhibitory impact of the aqueous extract on alpha amylase and alpha glucosidase enzyme activity in vitro and to evaluate its potential for enhancing insulin sensitivity *in vivo*. In the present study, the content of total phenols, flavonoids, tannins and saponins in AEFVC was determined. The results showed that AEFVC contains all of these metabolites but in different proportions. These results are in the same direction as those obtained by Lawan *et al.* (2008) who demonstrated that a preliminary phytochemical analysis on the methanolic extract of the bark of *F. vallis-choudae* revealed the presence of flavonoids, tannins and saponins (Adekunle *et al.*, 2005). Acarbose and AEFVC exhibited strong concentration-dependent inhibitory action in the  $\alpha$ -amylase and  $\alpha$ -glucosidase enzyme in vitro inhibition tests. In contrast to the  $\alpha$ -amylase enzyme, which had an  $IC_{50}$  of  $32.71 \pm 0.96$   $\mu$ g/mL, the extract showed the strongest potential for  $\alpha$ -glucosidase activity inhibition, with an  $IC_{50}$  of  $24.08 \pm 1.21$   $\mu$ g/mL. Overall, acarbose's inhibitory action was higher than the extract's. Nevertheless, there is not always a correlation between the equivalent *in vivo* activity and the *in vitro* inhibitory activity. Therefore, preclinical animal experiments must establish proof of concept. Doses of 220 and 440 mg/kg of EAFVC significantly reduced blood glucose levels 90 and 120 minutes after starch delivery in-vivo tests. Regarding the oral sucrose tolerance test, all EAFVC dosages examined at 60, 90, and 120 minutes showed a drop in postprandial

blood glucose. AEFVC seems to decrease postprandial hyperglycemia by delaying the quick digestion of starch and sucrose and extending the time it takes for carbohydrates to be absorbed.

Thus, the extract showed a significant inhibitory effect on the enzymes  $\alpha$ -glucosidase and  $\alpha$ -amylase both *in vitro* and *in vivo*. These results suggest that EAFVC may decrease postprandial blood glucose levels by inhibiting the activity of  $\alpha$  amylase and  $\alpha$ -glucosidase, which are important enzymes in the digestion of complex carbohydrates into absorbable monosaccharides in foods. Reduced postprandial hyperglycemia, improved altered metabolism of the small intestine, and inhibition of  $\alpha$ -glucosidase found in the small intestine's epithelium have all been demonstrated by inhibitors of digestive enzymes like acarbose (Sima and Chakrabarti, 2004). The persons with type 2 diabetes who have blood sugar levels that are only marginally higher than the threshold for diabetes are the ones who benefit most from these drugs.

Acarbose yielded outcomes in the current investigation that were nearly identical to those of the plant extract. This is consistent with research by Kamiyama *et al.* (2010), which demonstrated that acarbose would contain compounds that bind to  $\alpha$ -glucosidase and  $\alpha$ -amylase and have the same structure as the disaccharides and monosaccharides produced during the digestion of carbohydrates. This would allow the compounds to competitively inhibit pancreatic  $\alpha$ -glucosidase.

The free radical scavenging capacity of DPPH is widely used to analyse the antioxidant potential of foods and plants. In the present study, EAFVC showed a potential inhibitory effect against DPPH free radicals with an  $IC_{50} = 98.30 \pm 2.21$   $\mu$ g/mL. This value is higher than that obtained by Ali *et al.* (2012) in an anti-free radical test conducted on the methanolic extract of *F. vallis-choudae* bark. These authors obtained a value of 87.4  $\mu$ g/mL. This difference may be due to the type of solvent used to extract the bioactive compounds from a plant or the part used for the study. The FRAP assay is used as a good indicator for assessing the antioxidant potential of a plant or food. The FRAP antioxidant activity of the extract was evaluated in the present work. Indeed, the extract showed a significant FRAP antioxidant potential with an  $IC_{50}$  of  $95.90 \pm 3.30$   $\mu$ g/mL. Phenolic compounds in plant extracts are one of the major classes of chemicals functioning as principal antioxidants or free radical scavengers, according to earlier research by Lee *et al.* (2007). Our extract's different phenolic components may function as reducing agents, converting free radicals into stable molecules.

T2D associated with obesity is characterized by an increase in the concentration of glucose in the blood, following pancreatic  $\beta$ -cell dysfunction, an increase



in glucose production by the liver (gluconeogenesis and glycogenolysis) and a decrease in glucose uptake by peripheral tissues (Wellen and Hotamisligil, 2005). Under normal physiological conditions, patients who develop insulin resistance following obesity can increase insulin secretion and maintain glucose homeostasis for a long time, avoiding the development of diabetes (Marchetti *et al.*, 2006). Rats in the current study had higher blood sugar levels than the normal control after being fed a diet high in hog fat for 30 days. Gonzalez *et al.* (1989) demonstrated that a high-fat diet raises glucose production by decreasing insulin suppression and promoting gluconeogenesis, which raises plasma glucose levels. These findings are consistent with their findings. In a similar vein, untreated diabetic rats showed a markedly higher level of insulinemia than normal rats. It is commonly known that whole-body insulin resistance and beta cell failure are hallmarks of type 2 diabetes (Goossens, 2008). Furthermore, Despres and Lemieux (2006) found that insulin resistance in these various organs would be influenced by lipotoxicity, which is caused by an ectopic buildup of lipids in the liver, muscles, and heart.

All groups of treated T2DM rats showed decreased blood glucose and insulinemia after 28 days of co-treatment with EAFVC and metformin. This would indicate that insulin is sensitive to these distinct target cells. In fact, the reduction of insulin resistance at the level of target cells, including the liver, muscle, and adipose tissue, the stimulation of glycogenesis and glycolysis, and the inhibition of gluconeogenesis in the liver may all be associated with a plant's hypoglycemic effect (Singh *et al.*, 2012). Thus, the same mechanisms of action may be responsible for our extract's hypoglycemic effects. Diabetes is linked to abnormalities in lipoprotein and plasma lipid metabolism, which are typified by decreased HDL-c levels and increased CT, LDL-c, and TG concentrations. In both T1D and T2D, this dyslipidemia has a significant impact on the onset and progression of early atherosclerosis, which causes CVD. For diabetes people, cardiovascular disease is the leading cause of death (American Diabetes Association, 2005).

Rat models of diabetes that were experimentally induced by a diet high in pork fat (DT2) and, on the other hand, by an injection of STZ at a dose of 55 mg/kg (DT1) resulted in a significant drop in serum concentrations of C-HDL and IC and an increase in serum concentrations of CT, TG, LDL-c, and VLDL-c, as well as an increase in AI and CRI. In rats fed a diet high in pig fat for 28 days, the serum concentrations of CT and TG increased by around 75% and 80%, respectively, whereas the serum concentration of HDL-c significantly decreased, according to research by Vijaya *et al.* (2009). One possible explanation for the observed hyperlipidemia is that the buildup of fat in the liver led to an increase in the formation of acetyl Co-A, a precursor of cholesterol, in the liver cells. Saturated fatty acids make up 41.2% of bacon, monounsaturated fatty acids 48.9%, and

polyunsaturated fatty acids 9%. According to Baraas (1993), consuming saturated fats can raise total cholesterol levels and lower HDL-c, which will raise the ratio of CT to HDL-c. In the present study, the results showed after treatment with AEFVC a drop in the serum concentration of CT, LDL-c, TG, VLDL-c and a significant increase in the serum concentration of HDL-c.

The pathophysiology of several cardiovascular illnesses, such as diabetes and obesity, is influenced by reactive species. The current study measured the activities of CAT and SOD, GSH, and MDA levels in the liver, heart, and kidney of diabetic rats in order to assess the impact of EAFVC on oxidative stress markers. GSH plays a multifactorial role in the antioxidant mechanism. It is a direct scavenger of free radicals and participates in the regeneration of oxidized vitamin E. Therefore, changes in the redox state of GSH can be considered a particularly sensitive indicator of oxidative stress (Taleb-Senoucia *et al.*, 2009). In the present study, the results obtained showed a significant drop in hepatic, renal and heart GSH levels in all diabetic controls. Loven *et al.* (1986) found similar results and suggest that the decrease in the concentration of GSH in diabetic rats is probably due on the one hand to an increase in its use by liver and kidney cells, and on the other hand to a decrease of GSH synthesis or an increase in its degradation during oxidative stress caused by diabetes. On the other hand, our study revealed that treatment with EAFVC led to a significant increase in GSH in the liver, kidney. These results suggest that our extract was able to either increase glutathione biosynthesis or reduce oxidative stress, leading to a reduction in its degradation, or influence both mechanisms at the same time.

Reduced SOD and CAT activity may alter the equilibrium of antioxidant enzymes, resulting in inadequate antioxidant defenses against ROS and tissue damage (Sivajothi *et al.*, 2008). One of the most crucial antioxidant enzymes in the body's defense mechanism is SOD. It lessens the harmful effects of this free radical and other radicals produced by secondary reactions by catalyzing the dismutation of the superoxide anion  $O_2^-$ -hydrogen peroxide into  $H_2O_2$  (Yue *et al.*, 2003). However, this process can lead to lipid peroxidation if the  $H_2O_2$  is not broken down immediately (Taleb-Senoucia *et al.*, 2009). CAT is a ubiquitous enzyme and forms part of the important first line of antioxidant defence. It reduces  $H_2O_2$  to  $H_2O$  and  $O_2^-$  to prevent the induction of a SO state, maintain cell homeostasis and play an important role in the elimination of ROS. The decrease in SOD activity observed in these organs in diabetic rats could be due to glycation of the enzyme, which causes its inactivation and contributes to the worsening of oxidative damage caused by the overproduction of superoxide anion ( $O_2^-$ ) and hydroxyl radicals ( $OH^-$ ). The reduction in CAT activity in untreated diabetic rats compared with normal rats could be the result of inactivation of the enzyme by superoxide anion, by glycation or by both mechanisms at the same time. This also explains the

inability of SOD to protect CAT (Kakkar *et al.*, 1998). After treating diabetic animals with EAFVC, an increase in the activity of these two enzymes was observed in these organs. This increase suggests that this oxidative defence could be reactivated by the active principles present in EAFVC, which could cause an increase in detoxification capacity by improving the capture of free radicals. Lipid peroxidation is a key marker of oxidative stress and is determined by measuring MDA. The results obtained in our study showed an increase in the tissue concentration of MDA in the liver and kidney of diabetic controls. These results may be explained by lipid auto-oxidation, which is probably induced by STZ injection on the one hand, and by obesity, which is characterised by hypertriglyceridaemia and hypercholesterolaemia on the other (Saka *et al.*, 2011 ; Tchoubou *et al.*, 2023). On the other hand, the drop in MDA levels after treatment would suggest that EAFVC was able to protect liver and kidney tissues against oxidative stress and cytotoxic action induced by STZ, so it was able to improve the pathological state of diabetes by inhibiting lipid peroxidation. Furthermore, the reduction in lipid peroxidation in diabetic rats treated with the plant could be due to the increase in antioxidant status, as the aqueous extract of the plant exhibited high antioxidant activity by increasing the activity of SOD, CAT and GSH compared with the control groups. The pancreatic tissue of the normal rat presents a normal appearance, composed of solid parenchyma with a lobular structure separated by fibroadipous tissue. The abnormal state of the islets of Langerhans in IDC is the result of the effect of streptozotocin. Administration of AEFVC at different doses showed a moderate improvement in beta islet size compared with the untreated group. Previous studies by Alvarez *et al.* (2012) have reported that antioxidants help beta cells regenerate and protect them from the toxic effect of streptozotocin. Thus, thanks to its antioxidant capacity, AEFVC protects pancreatic beta islet cell from the cytotoxic effects of streptozotocin, and also leads to their regeneration.

## Conclusion

The study demonstrates that AEFVC exhibits significant antioxidant activity and effectively inhibits the enzymes  $\alpha$ -amylase and  $\alpha$ -glucosidase. Additionally, it enhances insulin sensitivity in rats subjected to a high-fat diet. These effects are attributed to the extract's rich phenolic content, including phenols, flavonoids, tannins, and saponins. These findings provide scientific support for the traditional use of AEFVC in managing type 2 diabetes and related metabolic conditions.

## Declaration

### Conflict of interest

Regarding the research, writing, and/or publication of this paper, the author or authors have disclosed no potential conflicts of interest.

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## Consent to participate

All participants agreed to consent to participate anonymously.

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NA.

## Authors Contribution

Design, collection, interpretation and analysis of data, final approval of study and article content and accountability for data integrity saw the participation of all authors. Kilenma Kolefer: Writing-Original draft, Investigation, Conceptualization, Development of research strategy. All authors read and approved the final manuscript.

## Ethical Approval

The Cameroon National Ethics Committee (ref. no. FWIRB 00001954) approved and examined all animal experiments before they were carried out.

## Abbreviation

AEFVC: Aqueous extract of the leaves of *Ficus vallis-choudae*

IDC: Insulin-resistant diabetic control

HDL-c: High-density lipoprotein cholesterol

LDL-c: Low-density lipoprotein cholesterol

VLDL-c: Very-low-density lipoprotein cholesterol

HOMA-IR: Homeostasis model assessment of insulin resistance

HOMA- $\beta$ : Homeostasis model assessment of  $\beta$ -cell function.

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