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Original Article

Crassocephalum crepidioides (Asteraceae) Benth S. Moore Leaves Fractions Attenuate Dyslipidemia and Atherogenic Indices in Diabetic Rats

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ABSTRACT

Background: Diabetes mellitus, a metabolic and endocrine disorder, is associated with an impaired lipid profile that can result in increased atherogenic indices. The effects of the aqueous and hexane fractions of *C. crepidioides* leaves on the lipid profile and atherogenic indices of diabetic rats were investigated in this study. **Materials and Methods:** Varied concentrations (50-200 mg/kg body weight) of the aqueous (CAF) and hexane (CHF) fractions of *C. crepidioides* were assayed against streptozotocin-induced diabetic rats. Histological examinations of the pancreas were carried out using hematoxylin and eosin staining procedures. Experimental rats were randomly divided into 9 groups of 6 rats each and orally treated for 14 days. **Results:** The tested concentrations (50, 100 and 200 mg/kg) of CAF and CHF significantly ($p < 0.05$) reduced plasma glucose (51.3-62.2%), plasma and liver triglycerides (up to 50.5% in plasma; 66.1% in the liver), total cholesterol (up to 49.0% in plasma; 35.3% in the liver), low-density lipoprotein-cholesterol (up to 96.0 and 91.0% in the plasma and liver), very low-density lipoprotein-cholesterol (up to 50.5% in plasma; 55.0% in the liver), and the atherogenic indices elevated by diabetes induction. The high-density lipoprotein-cholesterol concentrations were significantly increased (plasma: 73.60-127.60%; liver: 108.70-152.5%) in CAF and CHF-treated diabetic rats compared to the diabetic control. Histological examination showed improved tissue architecture in the pancreas of the diabetic-treated rats compared to the diabetic control. **Conclusion:** *C. crepidioides* leaf fractions possess hypolipidemic and anti-atherogenic activities. Therefore, the plant could be useful in managing diabetes, dyslipidemia, and cardiovascular conditions.

Keywords: Atherogenic indices, Diabetes mellitus, Dyslipidemia, Histological, Solvent fractions.

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INTRODUCTION

Diabetes, a metabolic and endocrine system malfunction, has gained a significant focus in global health, and its prevalence is continually rising in developing countries including Nigeria.[1] The World health organization projected that by 2025, diabetic incidents in the world would have risen to about 300 million.[2] However, the projected statistics were surpassed by 28.9% by 2014.[3] The incidence of diabetes is growing in Africa, and it has been predicted that the percentage increase in the burden of diabetes from the year 2019 to 2045 will be greatest in the African region; recording about 143%.[4] Similarly, there has been a constant increase in the occurrence of diabetes mellitus in Nigeria; as of 2019, 8.2 million Nigerians were estimated to have impaired glucose tolerance, with a predicted increase to 11.5 million by 2030.[4,5]

Diabetes affects metabolisms and modulations on pertinent biochemical parameters characterized by hyperglycemia,[6] dyslipidemia – hypertriglyceridemia, reduced High-Density Lipoprotein (HDL) - cholesterol concentration, Low-Density Lipoprotein (LDL),[7-9] modulated lipid profile with resultant changes in the atherogenic index, coronary heart disease,[10] continue to arouse research interests in a quest to finding phytoconstituents with potent chemical scaffolds to ameliorate the metabolic disorders resulting from diabetes. Previous studies indicate that elevation in blood and organ circulations of the atherogenic lipids emanate from increased synthesis of triglycerides that results from increased free fatty acid mobilization from the insulin-resistant adipose cells, and

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activation of Apolipoprotein B (ApoB) and VLDL-cholesterol production.[11] The elevated triglyceride levels are a result of increased production and decreased clearance of triglyceride-rich lipoproteins in both fasting and non-fasting states.[10,12] Increased production of VLDL as a main transporter of fasting triglycerides, is a significant characteristic of diabetes and insulin resistance.[13]

Streptozotocin (STZ), one of the diabetogenic compounds, induces diabetes mellitus in laboratory animals by obliterating the insulin-producing pancreatic beta-cells. STZ limits the generation of insulin and specifically destroys beta cells that produce insulin by causing necrosis, thereby compromising organ function.[14,15] Several successful treatments have been recorded for diabetes, but the adverse effects associated with synthetic drugs are rather creating fears than remedies. The existing therapies however are limited in efficacy, tolerability, and/or significant mechanism because of the accompanying side effects.[16] Hence, there has been a paradigm shift in research on alternative sources, especially of natural products origin for the management and treatment of diabetes mellitus.

Medicinal plants are a relevant source of novel therapeutic agents. The tropical rainforest of West Africa and other climate regions of the world are endowed with a plethora of medicinal plants which over the years have been explored for their biological importance. The extracts of these plants are rich in phytoconstituents that have been used by man as prophylactics or therapeutic agents in the treatment of various diseases.[17,18] The alteration of biochemical processes by these phytochemicals has been demonstrated to lower the risk of metabolic disorders in humans.

Crassocephalum crepidioides (Asteraceae) Benth S. Moore is an edible plant found in tropical and sub-tropical regions, including Nigeria. In Southwest Nigeria, it is called "ebolo," Akwa Ibom, and Edo, South-Southern Nigeria call it "mkpafit" and "Obuinenawa" respectively.[19] Several ethnomedical applications of *C. crepidioides* have been documented, including the treatment of acute hepatitis, boils, edema, fever, wounds, indigestion, and stomach ulcers.[20-23] *C. crepidioides* has been reported to possess various biological properties such as anti-bacterial, anti-diabetic, anti-helminthic, anti-inflammatory, antioxidant, and acetylcholinesterase inhibitory, anti-coagulant activities,[7,24-29] as well as cytoprotective, cancer chemopreventive effects,[30,31] and anti-tumor activity in mice through stimulating the generation of nitric oxide *in vivo*.[23]

Based on the reported use of *C. crepidioides* in herbal medicine and its efficacy in the treatment of various ailments as well as the reported antidiabetic activity,[7] it is expedient to evaluate the effect of the extract of the plant on lipid profile and atherogenic indices of diabetic rats. The information obtained may clarify and validate the plant potential in the treatment of diabetes and dyslipidemia, and may also show the possible effect on the tissue architecture of the pancreas. Therefore, the study was conducted to evaluate how the aqueous and hexane fractions of *C. crepidioides* leaf methanolic extract affect the lipid profile, atherogenic indices, and pancreas tissue histology of diabetic rats.

MATERIALS AND METHODS

Collection and identification of Plant

Fresh leaves of *C. crepidioides* were collected from Ijesa farm at Ilishan-Remo, Ogun State, Nigeria (6.8932° N, 3.7105° E). Identification and authentication of the plant were done by Mr. G. A. Ademoriyo, a botanist at the IFE herbarium, Obafemi Awolowo University, Ile-Ife, Nigeria. A voucher specimen was deposited with the registration number IFE 17634.

Preparation of extracts and phytochemical characterization

The collected plant samples were sorted to remove decayed leaves and unwanted materials. The extract and fractions were obtained as previously reported.[32] Briefly, the pulverized leaves were extracted with 70% methanol to get the crude methanol extract which was then subjected to solvent partitioning to obtain the Aqueous (CAF) and Hexane (CHF) fractions. The hexane fraction was analyzed for the phytochemical constituents using Gas Chromatography-Mass Spectrometry (GC-MS) and has been reported.[33]

Experimental Animals

For this investigation, Wistar albino adult male and female rats weighing 120–200 g were used-54 males and 12 females. The rats were brought to Babcock University's experimental animal facility from Ladoke Akintola University's animal breeding facility in Ogbomoso, Oyo State, Nigeria. They were kept in cages with a 12 hr light/dark cycle at room temperature and fed regularly with rodent pellets and provided free access to tap water during the 14 days acclimatization and study period. The experiment's animal care and handling procedures adhered to National Institutes of Health (NIH) animal care recommendations, and Babcock University Health Research Ethics Committee (BUHREC) ethical permission was acquired with Certificate No. BU/BUHREC436/17.

Acute toxicity test and Induction of Diabetes

The acute toxicity of the plant was assessed using the Lorke's method[34] according to the report of Elufioye and Onoja.[35] With a slight alteration, the Furman method[36] for experimental diabetes induction was carried out. The induction procedure was as previously reported.[25] Rats with sustained Fasting Blood Glucose (FBG) levels over 200 mg/dL were used for the experiment.

Experimental Design

The following oral treatments were given to the rats daily for 14 days as randomly divided into groups of 6 rats each:

Group 1: Normal control.

Group 2: Diabetic control.

Group 3-5: Diabetic rats + CHF (50, 100, and 200 mg/kg respectively).

Group 6-8: Diabetic rats + CAF (50,100 and 200 mg/kg respectively).

Group 9: Diabetic rats + metformin (100 mg/kg).[37]

*CHF: hexane fraction of *C. crepidioides.* CAF: aqueous fraction of *C. crepidioides.*

The animals were monitored and administration of plant extract was done orally, once daily using a gastric tube for 2 weeks. A gastric tube was used to administer plant extract orally once daily to the animals for two weeks, and they were kept under observation. On completion of administration, blood samples were drawn through a heart puncture under anesthesia, transferred to Lithium Heparin bottles, and spun for 15 min at 2500 g to produce plasma for the biochemical assays.

Tissues Preparation and Histopathological Examination

The rats were dissected, and the whole liver and pancreas organs were removed. A weighed portion was cut, rinsed in ice-cold 0.1 M PBS (pH 7.4), and homogenized to obtain a final solution of 10% (w/v) homogenates using Ultra Turrax homogenizer (IKA, Germany), after which the homogenates were spun at 12,000 *g* for 10 min to obtain the supernatants that were separated and refrigerated at 4°C.

The excised pancreatic tissues were washed in saline, and kept in labeled bottles containing 10% formalin. The tissues of the excised pancreas from the sacrificed rats were examined for histopathological changes using the hematoxylin and eosin staining method. Briefly, the pancreas tissues were processed and embedded in paraffin, and 5 µm sections were obtained and stained using hematoxylin and eosin. Observation of the histopathological changes and photomicrography of the sectioned and stained tissues was done using a light microscope with a fitted digital camera.

Glucose and lipid profile assay

During the investigation period, a One Touch digital® Glucometer (Accu-Chek) was used to measure the fasting blood glucose level. Using the Randox glucose (GOD-PAP) reagent, the plasma glucose level was determined following the administration of the plant samples and standard drugs. The lipid profile (Total Cholesterol (TC), Triglycerides (TG), and High-Density Lipoprotein-cholesterol (HDL-c) concentrations) of the plasma and liver homogenates from experimental animals were determined with Randox assay kits (Randox Laboratories Limited, UK). Low-Density Lipoprotein-cholesterol (LDL-c) and Very Low-Density Lipoprotein-cholesterol (VLDL-c) concentrations were estimated[38] as stated in the commercial kit's instructions. Atherogenic indices; Atherogenic Coefficient (AC), Atherogenic Index of Plasma (AIP), and Coronary Risk Index (CRI). were calculated using the formula:

$$AC = \frac{TC - HDL - c}{HDL - c} \quad [39]$$

$$AIP = log\frac{TG}{HDL - c} \quad [40]$$

$$CRI = \frac{TC}{HDL - c} \quad [41]$$

Statistical analysis

Descriptive analysis was done using GraphPad Prism 7.0 for Windows (GraphPad Prism Software, San Diego, CA, USA). The arithmetic mean ± standard error of the mean is used to express the results. By using one-way Analysis of Variance (ANOVA) and Tukey *post hoc* tests, significant differences were determined; $p < 0.05$ was considered statistically different for mean differences.

RESULTS

Acute toxicity

Rats administered the leaf extract and fractions up to 5000 mg/kg did not experience any deaths, fatalities, or unusual behavior.

Identified compounds from *C. crepidioides* leaf with hypolipidemic activity

The findings of the phytochemical analysis of the *C. crepidioides* leaf hexane fraction have previously been reported.[33] However, some compounds identified with potential hypolipidemic activities based on literature[42] are listed in Table 1.

Plasma glucose changes in diabetic and normal control rats treated with various concentrations of *C. crepidioides* leaf fractions

The variations in plasma glucose levels of diabetic rats treated with various doses of CAF and CHF are shown in Figure 1. The results showed a significant ($p < 0.001$) increase in plasma When compared to the normal control group (57.23 ± 8.39 mg/dL), the results revealed a significant ($p < 0.001$) increase in the levels of plasma glucose in the diabetes control group (181.60 7.18 mg/dL). Plasma glucose concentrations were significantly ($p < 0.001$) reduced in diabetic rats treated with *C. crepidioides* leaf fractions compared to the diabetic control, but there were no significant ($p > 0.05$) differences in glucose concentrations among the diabetic groups given different concentrations (50, 100 and 200 mg/kg) of the fractions (CAF and CHF) and the normal control group. Diabetic rats administered with metformin likewise showed a

significant reduction in their blood sugar (from 181.60 mg/dL to 92.22 mg/dL; Figure 1).

Effects of *C. crepidioides* fractions on the Plasma Lipid profile of the Experimental rats

Figures 2 and 3 depict the plasma lipid profiles of normal rats and diabetic rats given varied concentrations of *C. crepidioides* leaf fractions. When compared with the normal control group, there were significant ($p < 0.05$) increases in the plasma concentrations of TG, TC, VLDL-c, and LDL-c in the diabetic control group (Figure 2). However, in diabetic groups administered with CAF and CHF, these lipid concentrations were significantly reduced. The plasma HDL-c concentration was significantly lower in the diabetic control group in contrast with the normal control and diabetic rats treated with the plant fractions (Figure 3).

Plasma TG and TC

TG level was significantly ($p < 0.001$) elevated in the STZ-induced diabetic rats (128.0 ± 16.16 mg/dL) compared to the normal control group (72.60 ± 3.90 mg/dL). Treatment with varied concentrations (50-200 mg/kg body weight) of CAF and CHF showed significant ($p < 0.05$) dose-dependent reduction of plasma TG levels in the groups with CHF 100 mg/kg group having the lowest concentration of 63.33 ± 7.96 mg/dL.

Plasma TC level was increased significantly ($p < 0.01$) in the diabetic control group (126.20 ± 17.74 mg/dL) in contrast with normal rats (65.80 ± 9.38 mg/dL). The observed elevation was counteracted in CAF and CHF-treated groups with values ranging from 64.4 to 76.8 mg/dL. A similar reduction occured in diabetic rats treated with Metformin with a recorded value of 64.68±5.80 mg/dL (Figure 2).

Plasma HDL-c, VLDL-c, and LDL-c

The plasma HDL-c concentration was significantly reduced in diabetic control rats compared with the normal rats (34.20 ± 2.72 mg/dL against 25.00 ± 1.79mg/dL). Administration of different concentrations of CAF and CHF to the experimental rats caused elevations in their HDL-c levels. Varying the concentration of the CHF showed no significant difference in HDL-c concentrations among treated groups, but the CAF 100 mg/kg gave an HDL-c value (56.91 ± 3.90 mg/dL) that was significantly higher than that of 50 mg/kg and 200 mg/kg treated groups (43.41 ± 4.90 and 47.25 ± 1.04 mg/dL, respectively). An increased concentration (38.30 ± 3.53 mg/dL) was also recorded in the metformin group (Figure 3).

The plasma VLDL-c concentration was significantly ($p < 0.05$) elevated in the diabetic control group (25.60 ± 2.20 mg/dL) compared to the normal rats (14.52 ± 1.14 mg/dL). Treatment with different concentrations of CAF and CHF showed a significant reduction in VLDL-c concentrations in the rats with the CAF and CHF 100 mg/kg having the most significant reduction with values of 12.67± 1.59 and 13.58 ± 0.35 mg/dL, respectively. Similarly, there was a significant ($p < 0.0001$) increase in plasma LDL-c concentration of the diabetic control group (75.60 ± 2.20 mg/dL) compared with the Normal control group (17.08 ± 2.52 mg/dL). The observed elevation was countered in the CAF and CHF-treated rats in a concentration-dependent manner with the highest reduction recorded in CHF 100 mg/kg group (3.00 ± 0.23) (Figure 3).

Effects of *C. crepidioides* leaf fractions on Liver Lipid profile of the Experimental rats

Figures 4 and 5 show the liver lipid profile of normal control and diabetic rats administered with *C. crepidioides* leaf fractions. The diabetic control group's liver TG, TC, VLDL-c, and LDL-c concentrations were markedly ($p < 0.05$) higher than those of the normal rats. Treatment of the diabetic rats with varied doses (50-200 mg/kg) of CAF and CHF significantly lowered the lipids concentrations. As compared to normal rats, there was a notable decrease in the liver HDL-c concentration in diabetic rats; however, CAF and CHF treatment significantly ($p < 0.05$) increased the HDL-cholesterol concentrations.

Liver TG and TC

Liver TG values were significantly ($p < 0.001$) increased in diabetic rats (232.20 ± 24.09 mg/dL) compared to the normal rats (78.20 ±

Table 1: GC-MS identified compounds in CHF with potential hypolipidemic activity.

Peak No.	Compound	Molecular Formula	Retention time (min)	Activity
112	Hexadecanoic acid	$C_{17}H_{34}O_2$	26.70	Hypocholesterolemic, antioxidant, anti-androgenic, 5-alpha reductase inhibitor, hemolytic.[42]
115	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	27.25	Hypocholesterolemic, anti-inflammatory, antioxidant, anti-androgenic, hemolytic, 5-alpha reductase inhibitor.[42]
122	Alpha-linolenic acid (9,12,15-Octadecatrienoic acid)	$C_{18}H_{30}O_2$	29.40	Hypolipidemic, anti-inflammatory, anti-aggregant, anti-leukotriene, anti-prostatic, immunostimulant, vasodilator, 5- alpha reductase inhibitor.

Table 2: Effects of *C. crepidioides* leaf fractions on Atherogenic indices of Diabetic rats.

Groups	AIP	AP	CRI
Normal Control	0.24 ± 0.01^{b}	1.01 ± 0.01^{d}	2.01 ± 0.20^{b}
Diabetic control	0.55 ± 0.03^{c}	3.44 ± 0.04^{f}	4.44 ± 0.13^{c}
Diabetic + CHF (50 mg/kg)	0.16 ± 0.02^{b}	0.52 ± 0.02^{b}	1.52 ± 0.06^{b}
Diabetic + CHF (100 mg/kg)	0.04 ± 0.01^{a}	0.27 ± 0.01^{a}	1.27 ± 0.05^{a}
Diabetic + CHF (200 mg/kg)	0.22 ± 0.02^{b}	0.54 ± 0.12^{b}	1.54 ± 0.08^{b}
Diabetic + CAF (50 mg/kg)	0.26 ± 0.04^{b}	1.25 ± 0.22^{e}	2.24 ± 0.17^{b}
Diabetic + CAF (100 mg/kg)	0.09 ± 0.01^{a}	0.30 ± 0.01^{a}	1.30 ± 0.04^{a}
Diabetic + CAF (200 mg/kg)	0.23 ± 0.05^{b}	0.55 ± 0.08^{b}	1.55 ± 0.10^{b}
Diabetic + Metformin	0.30 ± 0.09^{b}	0.69 ± 0.13^{c}	1.69 ± 0.11^{b}

Results are mean ± SEM of duplicate determination ($n = 4$). Significant difference at $p < 0.05$. Mean values with different superscript letters are significantly different within groups.AIP: Atherogenic index of plasma; AC: Atherogenic coefficient; CRI: Coronary risk index; CHF: Hexane fractionof *C. crepidioides*; CAF: Aqueous fraction of *C. crepidioides*.

5.70 mg/dL). However, TG concentration in experimental groups was shown to be considerably ($p < 0.01$) decreased by varying doses of CAF and CHF. There were no differences in the liver TG levels among the treatment groups. Significantly higher ($p < 0.01$) TC concentration was observed in diabetic control rats (133.10 ± 15.09 mg/dL) compared with the normal rats (66.20 ± 8.52 mg/dL). However, significant reductions ($p < 0.05$) in TC concentrations (73.22–97.22 mg/dL) were seen in diabetic-treated groups compared to diabetic control (Figure 4).

Liver HDL, VLDL and LDL-c

A significant ($p < 0.05$) reduction in liver HDL-c concentration was recorded in diabetic control rats in contrast with normal control (32.80 ± 2.63 mg/dL to 20.80 ±1.02 mg/dL). Administration of different concentrations of CAF and CHF to diabetic rats markedly increased the liver HDL-c levels of the experimental rats at all test concentrations with the highest increase seen in CHF 100 mg/kg, CAF 50, and 100 mg/kg groups (52.53 ± 0.98, 48.78 ± 5.56, and 50.74 ± 6.52 mg/dL, respectively).

Significantly increased ($p < 0.0001$) liver LDL-c level was observed in diabetic control rats (39.56 ± 3.32 mg/dL) compared to the normal control rats (17.76 ± 2.75 mg/dL). However, appreciably lowered LDL-cholesterol concentrations (3.70 – 17.67 mg/dL) compared to the diabetic control group were recorded in groups administered with different CAF and CHF concentrations. The lowest concentration of 3.70±0.02 mg/dL was recorded in CHF 100 mg/kg group (Figure 5). VLDL-c concentration was elevated in the diabetic control group (44.64 ± 4.82 mg/dL) than the normal rats (15.64 ± 1.14 mg/dL). Treatment of diabetic rats with plant leaf fractions significantly lowered the VLDL-c levels. There was no significant difference in values recorded for normal control and diabetic rats treated with varying doses (50,100 and 200 mg/kg) of CAF and CHF as well as Metformin (Figure 5).

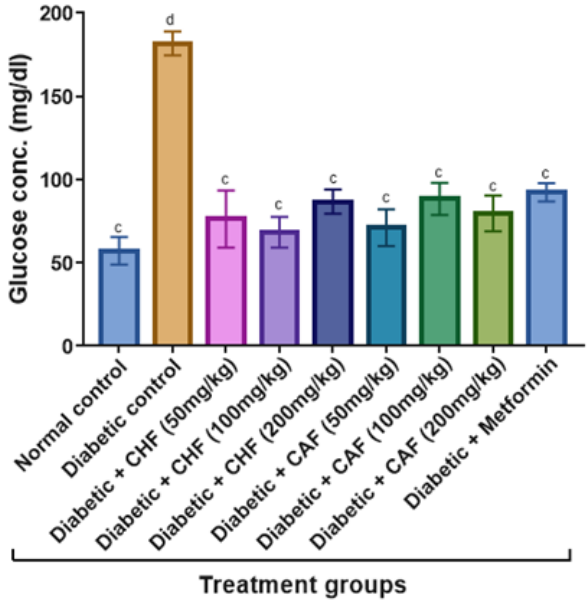

Figure 1: Changes in Plasma glucose concentrations of Diabetic rats treated with different concentrations of CAF and CHF.

Bars with different letters are significantly ($p < 0.0001$) different; $n = 4$. Diabetic rats were treated with varying concentrations (50, 100 and 200 mg/kg) of the Aqueous and Hexane fractions of *C. crepidioides*. CHF = hexane fraction of *C. crepidioides*; CAF = aqueous fraction of *C. crepidioides*.

Diabetic rats were treated with varied concentrations (50,100 and 200 mg/kg) of the Aqueous and Hexane fractions of *C. crepidioides*.

Effects of *C. crepidioides* leaf fractions on Atherogenic indices Experimental rats

The Atherogenic indices of normal control, diabetic control, and diabetic-treated rats are shown in Table 2. The AIP, AC, and CRI were significantly ($p<0.05$) higher in diabetic control rats compared to the normal rats. These values were significantly reduced to different extents in the treatment groups with the

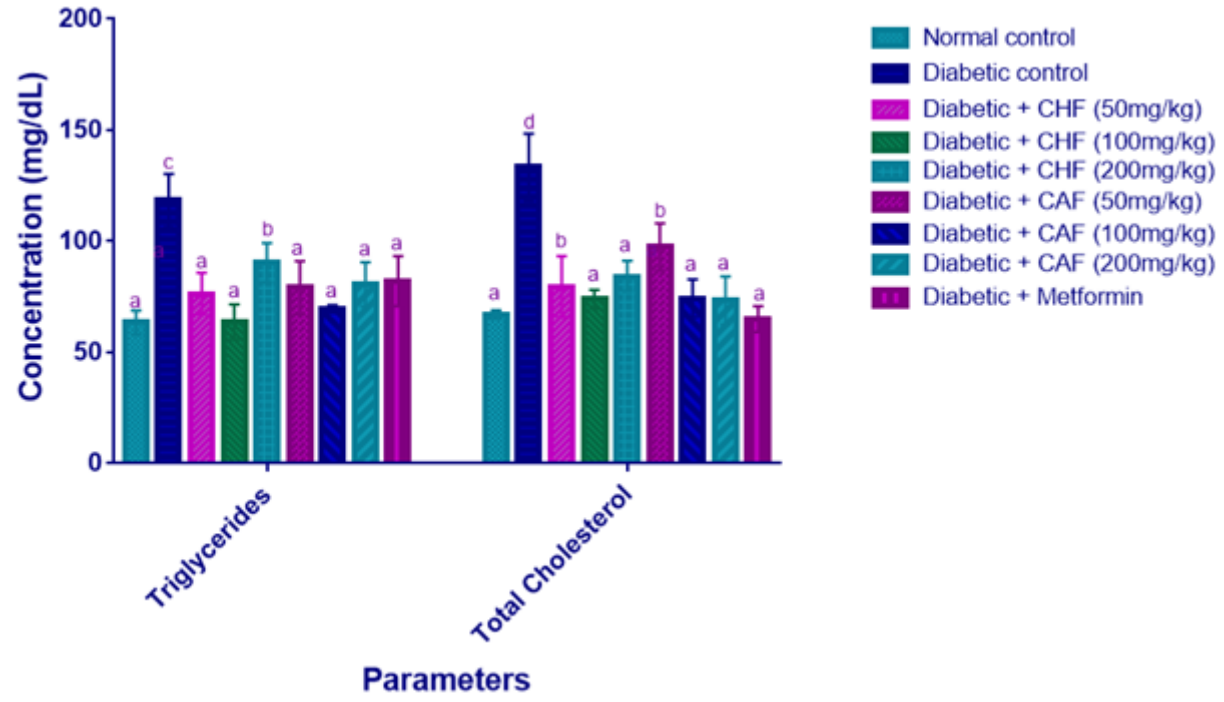

Figure 2: Changes in Plasma Triglycerides and Total cholesterol concentrations of Diabetic rats treated with different concentrations of CAF and CHF.

Bars with different letters are different from one another (n=4); b is significantly higher than a at $p < 0.05$; c and d are significantly higher than a at $p < 0.01$; d is significantly higher than b and c at $p < 0.05$.
Diabetic rats were treated with varying concentrations (50, 100 and 200 mg/kg) of Aqueous (CAF) and Hexane (CHF) fractions of *C. crepidioides*.

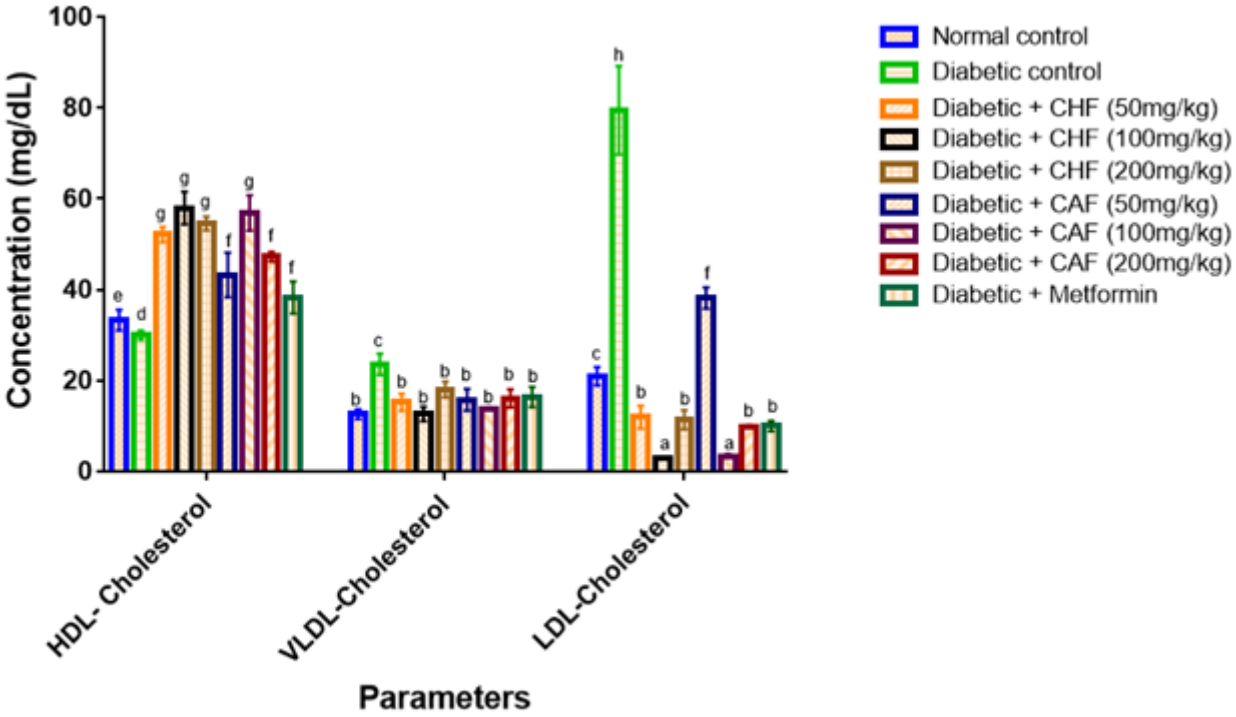

Figure 3: Changes in Plasma HDL-, VLDL- and LDL-Cholesterol concentrations of Diabetic rats treated with different concentrations of CAF and CHF.

Bars with different letters are significantly different from one another (n=4); b and c are significantly higher than a at $p < 0.001$. d, e, f, g and h are significantly higher than a at $p < 0.0001$. b is significantly lower than c, d and e ($p < 0.05$). e) f, g and h are significantly higher than b and c at $p < 0.001$. d is significantly lower than e, f and g ($p < 0.01$)
Diabetic rats were treated with varying concentrations (50, 100 and 200 mg/kg) of the Aqueous and Hexane fractions of *C. crepidioides*.

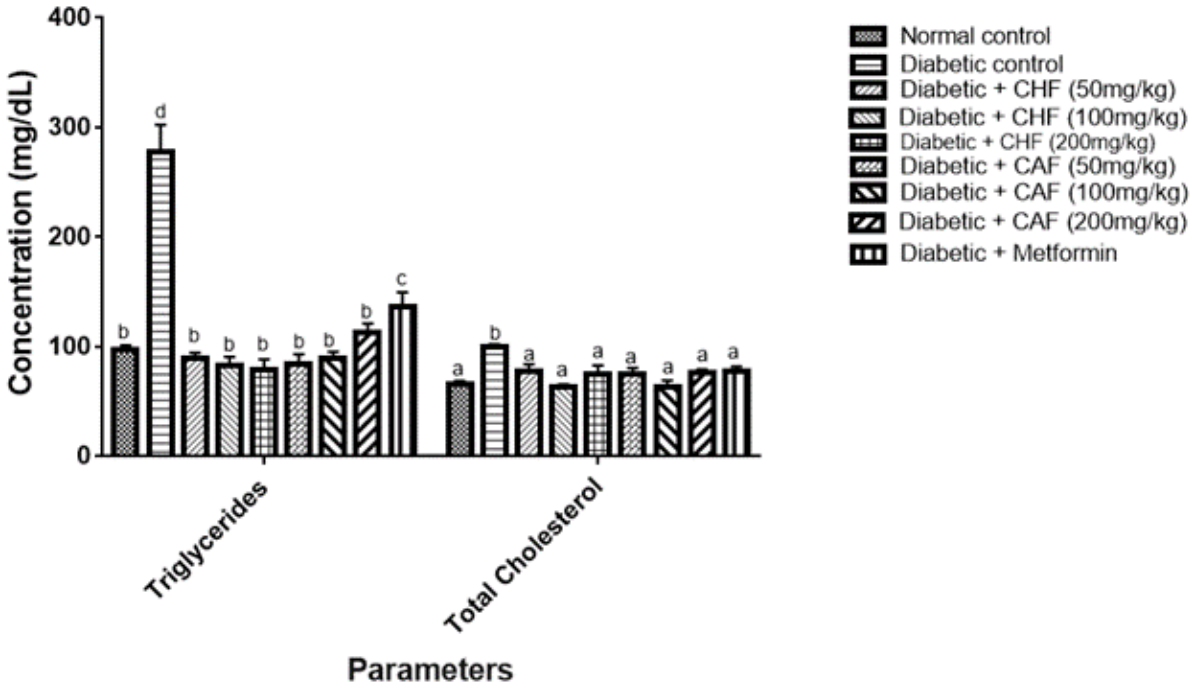

Figure 4: Changes in Liver Triglycerides and Total cholesterol concentrations of Diabetic rats treated with different concentrations of CAF and CHF.

Bars with different letters are significantly different from one another (n=4); b and c are significantly higher than a at $p < 0.05$. d is significantly higher than a and b at $p < 0.0001$.
Diabetic rats were treated with varying concentrations (50, 100 and 200 mg/kg) of the Aqueous and Hexane fractions of *C. crepidioides*.

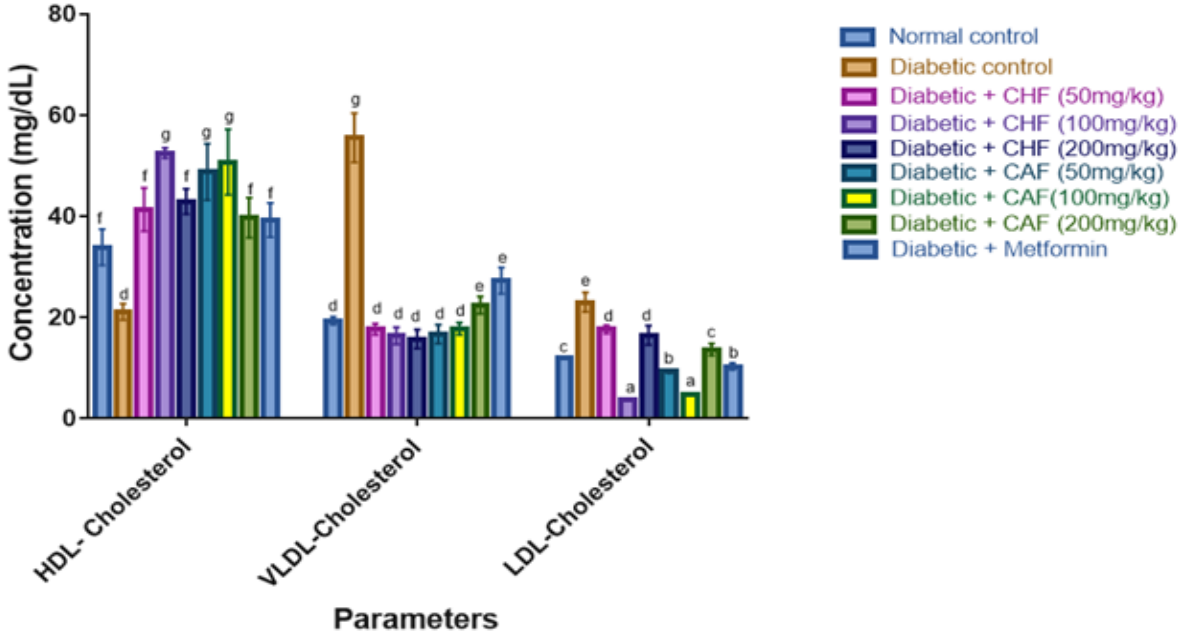

Figure 5: Changes in Liver HDL-, VLDL-, and LDL-cholesterol concentrations of Diabetic rats treated with different concentrations of CAF and CHF.

Bars with different letters are significantly different from one another (n=4). b, c and d are significantly higher than a at $p < 0.01$. e, f, g and h are significantly higher than a at $p < 0.0001$. d and e are significantly higher than c at $p < 0.05$. f and g are significantly higher than d and e at $p < 0.001$. g is significantly higher than f at $p < 0.05$. Diabetic rats were treated with varied concentrations (50, 100 and 200 mg/kg) of the Aqueous and Hexane fractions of *C. crepidioides*.

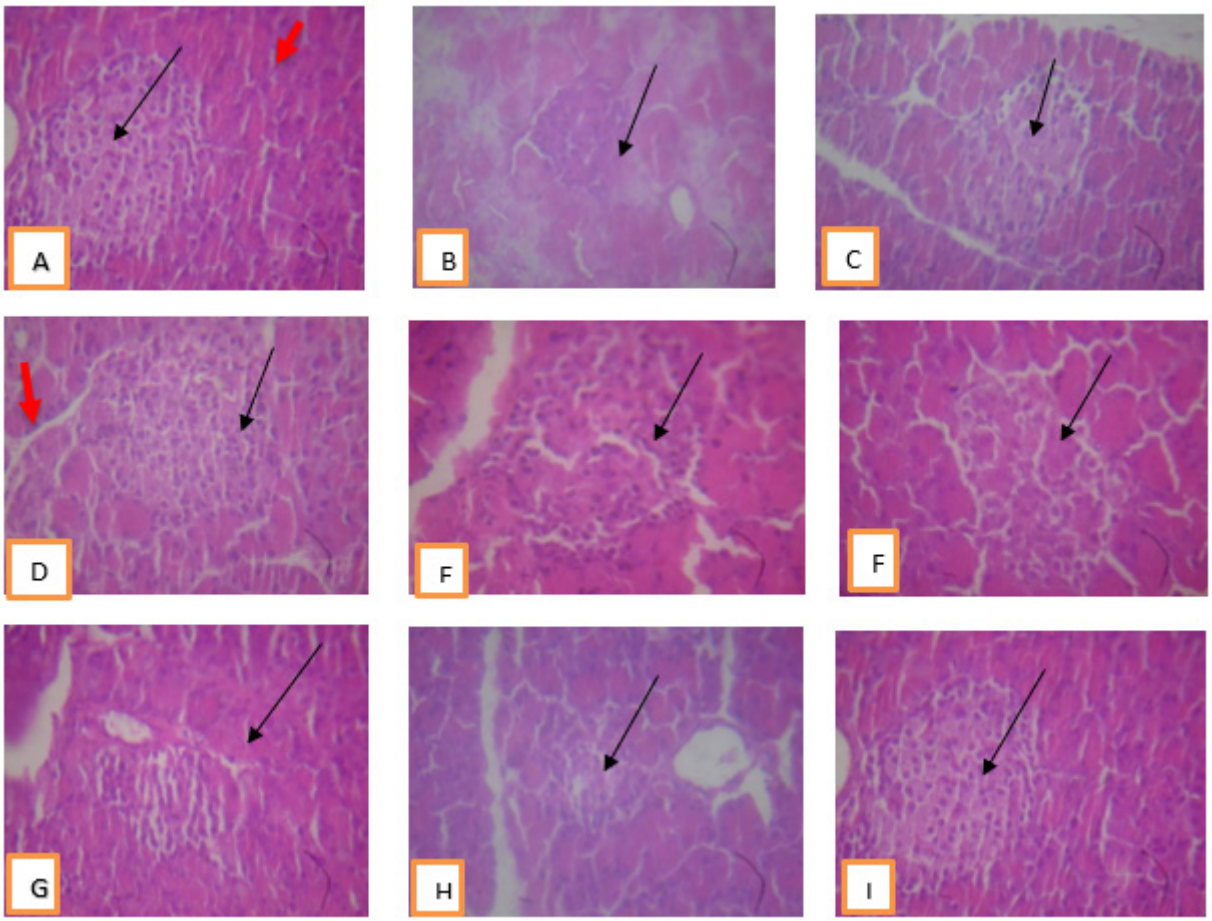

Figure 6: Photomicrographs of the pancreatic tissues: A: Normal control, B: Diabetic control, C-E: Diabetic rats given CHF (50, 100 and 200 mg/kg respectively), F-H: Diabetic rats given CAF (50, 100 and 200 mg/kg respectively), I: Diabetic rats given Metformin (100 mg/kg). H and E staining, Objective magnification: x 40.

lowest mean values (0.04 ± 0.01 for AIP; 0.27 ± 0.01 for AC; and 1.27 ± 0.05 for CRI) recorded in CHF 100 mg/kg group.

Histopathology of the pancreas of Normal control, Diabetic control, and Diabetic rats treated with *C. crepidioides* leaf fractions

The pancreatic tissues' photomicrographs of normal control, STZ-Diabetic control, and rats treated with different concentrations of CAF and CHF are shown in Figure 6. In the normal control rats (A), the histological section revealed normocellular islets (black arrow) surrounded by normal-appearing exocrine acini (red arrow); no necrosis was visible. Diabetic control rats showed substantial damage to the islet of Langerhans and a shrunken islet (black arrow) with severe necrosis surrounded by normal pancreatic acini (B). In diabetic rats treated with CAF and CHF at various concentrations (50, 100, and 200 mg/kg; C-H) and 100 mg/kg Metformin (I), partial improvement of the normal cellular population to varying degrees and expanded size of β-cells were seen. Improvement of pancreas architecture closely equivalent to normal was seen in the diabetic group given CHF 100 mg/kg (D) and Metformin (I).

DISCUSSION

Premature and extensive atherosclerosis is one of the main complications of diabetes mellitus. Systemic or local abnormalities in insulin may cause hyperglycemia as well as altered lipid metabolism and vascular wall function.[43] Lipid profile and atherogenic indices are important predictors for metabolic disorders such as dyslipidemia, atherosclerosis, hypertension, and cardiovascular diseases.[44]

The present study evaluated the effects of the aqueous and hexane fractions of *C. crepidioides* leaf methanol extract on the atherogenic indices, lipid profile, and histology of the pancreatic tissue of diabetic Wistar rats. The findings from this study agree with an earlier report in which methanol extract of *C. crepidioides* showed hypoglycemic effects in Alloxan-induced diabetic rats.[7]

By exerting a particular cytotoxic effect on pancreatic beta cells, STZ induces diabetes mellitus. It inhibits insulin production and selectively destroys the insulin-producing beta cells of the pancreas, thus affecting endogenous insulin release and increasing blood glucose levels.[14,15] A reduction in insulin release caused by the death of pancreatic β -cells may be the source of

observed increase in fasting blood glucose levels following STZ induction. The results of this investigation showed that various concentrations of the aqueous and hexane fractions of *C. crepidioides* effectively reversed the effect of STZ induction on elevated blood glucose. For improved insulin production, *C. crepidioides* fractions may act by regenerating beta cells; or alternatively, prevent cell death, lessen necrosis, and mitigate the effects of oxidative stress on the pancreatic β -cells. The plant's ability to lower blood sugar levels may be achieved by inducing the islets of Langerhans' surviving or regenerating cells to produce more insulin from the pancreas,[45] which would boost glucose utilization in the experimental rats.

The elevated TG, TC, LDL-c, VLDL-c, and lowered HDL-c observed in diabetic rats agree with the reports that Diabetes is accompanied by dyslipidemia.[14,46] The unchecked action of the adipose tissue hormone-sensitive lipase unchecked may result in increased mobilization of peripheral fat depots and a boost in the production of TG-rich lipoprotein particles in the liver. This may account for the significant hyperlipidemia observed in STZ-induced diabetic rats.

In mammalian cells, insulin reduces the activity of hormone-sensitive lipase, which catalyzes the mobilization of free fatty acids from stored triglycerides, stimulating the de novo production of fatty acids in the liver and reducing lipolysis in adipose tissue. As a result of increased production and lower clearance of TG-rich lipoproteins (Chylomicrons, VLDL, and LDL) in a diabetic condition, impaired insulin secretion or activity raises triglyceride levels.[10] Moreover, the rate of fatty acid transport into muscle is accelerated in diabetes, which may lead to an excess buildup of intracellular lipid metabolites that disrupt insulin signaling.[47]

When compared to diabetic control rats, the plasma and liver TG, TC, LDL-c, and VLDL-c concentrations of diabetic rats treated with *C. crepidioides* fractions were much lower and the plasma and liver HDL-c concentrations were significantly higher. This may be caused by a decreased hepatic triglyceride synthesis and/or decreased lipolysis as a result of potential insulin production stimulation by *C. crepidioides*. A favorable risk factor for atherosclerosis is indicated by the considerable decrease in HDL-c in diabetic rats, whereas this atherogenic risk is mitigated by the significant increase in HDL-c in rats treated with *C. crepidioides*.

The atherogenic indices (including AIP, AC, and CRI) are the critical indices that can be used for cardiovascular risk estimation. Abnormality in lipid profile increases the risk of atherosclerosis and coronary heart disease. AIP, most especially, has been reported to be strongly correlated with cardiovascular risks.[48] The estimated atherogenic indices were found to be significantly lowered in diabetic rats treated with *C. crepidioides* leaf fractions. The hexane fraction of *C. crepidioides* was previously characterized by GC-MS, and it identified the presence of n-hexadecanoic acid, hexadecanoic methyl ester, and alpha-linolenic acid with lipid-lowering effects.[33]

Histopathological analysis of the pancreatic tissues of the experimental rats revealed that the islet of Langerhans of the STZ-induced rats developed degenerative changes that led to shrinkage, which decreased the number of islet cells compared to the normal control rats. Similar to Li *et al*., [49] who performed a morphometric analysis of the islet endocrine cells in STZ-induced type I diabetes, reported that the area of the islet cells decreased by up to 35%, the number of insulin-positive beta cells gradually decreased, and the number of alpha cells increased by 2–3 times per islet area. The islet morphology and cellular population improved to diverse degrees after treatment with various concentrations of *C. crepidioides* leaf fractions, with the most notable improvement shown in diabetic rats treated with 100 mg/kg CHF.

CONCLUSION

C. crepidioides leaf fractions possess hypolipidemic and anti-atherogenic properties in diabetic rats. Alterations in lipid profile in diabetes mellitus predispose to an increased risk of coronary heart disease, thus a lowered blood atherogenic lipid profile should be regarded as beneficial in the long-term prognosis of diabetes mellitus. *C. crepidioides* leaves could be thus useful in controlling the development of dyslipidemia and cardiovascular complications in the diabetic state. The plant could be a source of biopharmaceutical agents and nutraceuticals for treating and managing diabetes, hyperlipidemia, and cardiovascular diseases.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Conceptualization: FDO, ORO. Investigation: OOA. Data curation: OOA, FDO, EEO. Original draft preparation: OOA, EEO. The manuscript was reviewed and approved by all authors.

ETHICAL APPROVAL

All authors hereby declare that "Principles of Laboratory Animal Care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approval was given by Babcock University Health Research Ethics Committee (BUHREC) with Certificate No. BU/BUHREC436/17.

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