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Therapeutic potentials of aqueous extract of *Costus afer* leaves on gastrointestinal function: A study on metabolic and hepatic changes in streptozotocin-induced type II diabetic rats

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Abstract

Introduction: Diabetes mellitus is a group of metabolic disorder in which there is high blood sugar over a prolonged period of time. This study aimed to compare gastrointestinal function and hepatic changes in diabetic rats treated with aqueous extract of *Costus afer* leaves, evaluate the effect of aqueous extract of *C. afer* leaves on liver enzymes in Wistar rats.
Methods: Thirty-five male rats were grouped in 5 groups of 7 rats. To induce diabetes, 4mg/ml of streptozotocin, was injected at 50mg/kg body weight to animals in groups II, IV and V. Oven-dried *C. afer* leaves were crushed into powdered, and packaged separately and used to produce aqueous extract for the study. The extract was administered at a dosage of 800mg/kg body weight daily to rats in groups III and V. After 28 days, the animals were sacrificed and parameters were analyzed. A stock concentration of 20mg/ml of metformin was administered at a dose of 200mg/kg of body weight to the animal using oral gavage to group IV animals only. Extracted serum was used for Alkaline phosphatase (ALP), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) analysis.
Results: Treatment with *C. afer* alone (5.62±0.21), and in combination with metformin significantly reduced blood glucose compared to the untreated diabetic group (9.82±0.76). Final body weight was significantly reduced in diabetes mellitus group (DM) and DM+ Metformin (MTF) groups but improved with *C. afer* and DM+*C. afer*. AST, ALT, and ALP were significantly elevated in DM rats, indicating liver dysfunction. However, treatment with *C. afer*, especially in combination with metformin, significantly restored these enzymes toward normal levels.
Conclusion: Findings support the antidiabetic, gastroprotective, and hepatoprotective efficacy of *C. afer* aqueous extract.

Key words: Costus afer, Diabetes mellitus, Gastrointestinal function, Hepatic changes, Metformin

Introduction

The rapid increase of diabetes mellitus (DM) worldwide has established it as an epidemic in the 21st century.[1] Diabetes mellitus, commonly referred to as diabetes is defined by the World Health Organization as a group of metabolic disorder in which there is high blood sugar over a prolonged period of time.[2] Symptoms of high blood sugar include frequent urination (polyuria), increased thirst (polydipsia) and increase hunger (polyphagia).[2] Diabetes mellitus is classified into three broad categories, type I, II, and gestational diabetes.

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Type I Diabetes mellitus is known as insulin dependent Diabetes mellitus which results from loss of insulin production by the pancreas, while type II Diabetes mellitus is characterized by insulin resistance and may also be combined with relatively reduced insulin secretion. The third type, gestational diabetes involves a combination of inadequate insulin secretion and responsiveness and occurs mostly in pregnant women.[2] For the purpose of this study, emphasis would be laid on type II diabetes mellitus.

The prevalence of DM is currently on a rise. In 2014, 8.5% of adults aged 18 years and older had diabetes. In 2019, diabetes was the direct cause of 1.5 million deaths and 48% of all deaths due to diabetes occurred before the age of 70 years. Another 460,000 kidney disease deaths were caused by diabetes, and raised blood glucose causes around 20% of cardiovascular deaths. From 2000 to 2019, there was a 3% increase in age-standardized mortality rates from diabetes. The number of people suffering from the disease worldwide is increasing at an alarming rate with a projected 366 million peoples likely to be diabetics by the year 2030 as against 191 million estimated in 2000.[3]

Diabetes mellitus is regarded as a systemic disease that may affect many organs system and the gastrointestinal (GI) system is no exception. GI sign and symptoms are common in diabetes mellitus and usually attributed to autonomic neuropathy.[4] These GI symptoms affect quality of life adversely and represent a substantial cause of morbidity in patients with diabetes.[5] Statistical reports have shown that as much as 75% of patients visiting diabetic clinics will report gastrointestinal symptoms ranging from diabetic gastroparesis, ulcers, early satiety, esophageal reflux, constipation, abdominal pains, nausea, vomiting and diarrhea. Early identification and appropriate management of GI complications are important for improving both diabetic care and quality of life.[6] Some plants have been used to manage diabetes mellitus, one of such plants is *C. afer*.

The phytochemical analysis of the leaves of *C. afer* shows the presence of alkaloids, phenols, saponins, triterpenes, tannins and glycocides.[7] *C. afer* acts as an antidiabetic agent via biochemical mechanisms including restitution of pancreatic β-cell function, amelioration of insulin resistance by sensitizing receptors, inhibition of liver gluconeogenesis, enhanced glucose absorption and inhibition of α-amylase and α-glucosidase activities. The leaves contain several bioactive compounds with diosgenin and aferosides A, B and C, named as the most likely compounds responsible for the antidiabetic properties of *C. afer*.[8] There is limited data on the therapeutic effects of aqueous extract of *C. afer* leaves on gastrointestinal and hepatic parameters in Wistar rats. This study therefore compared the gastrointestinal function and hepatic changes in streptozotocin-induced type II diabetic rats, treated with aqueous extract of *C. afer* leaves.

Methodology

Experimental animals and maintenance

Thirty five (35) male rats weighing between (160 – 200g) were purchased from the animal house of the Faculty of Basic Medical Sciences, College of Medicine, University of Calabar, Cross River State, Nigeria. The animals were allowed for two weeks to acclimatization in customized cages, and were fed with standard rat pellet, while allowed free access to clean water. The animals were randomly placed in 5 groups of 7 rats each (Table 1). Ethical Approval was obtained from the animal ethical committee of the Faculty of Basic Medical Science, University of Calabar, Calabar.

Table 1: Experimental design and animal grouping

S/N	Groups	No. of rats	Treatment regimen
1.	Control group	7	Normal rat feed + drinking water
2.	Diabetic group	7	Normal rat feed + drinking water + Streptozotocin
3.	*C. afer* group	7	Normal rat feed + drinking water – *C. afer*
4.	Diabetes + Metformin group	7	Normal rat feed + drinking water + Streptozotocin + Metformin
5.	Diabetes +*C. afer* group	7	Normal rat feed + drinking water + Streptozotocin + *C. afer*

Determination of blood glucose level

Initial blood glucose was measured in experimental animals via tail vein puncture using a lancet. A glucometer strip (Model: Finetest) was used to analyze a drop of blood. This provided a reading of the blood glucose levels.

Experimental induction of diabetes mellitus

After the period of acclimatization, immediately prior to injection, 4mg of streptozotocin (STZ) was dissolved in 50Mm sodium citrate buffer (pH 4.5) to a final concentration of 4mg/ml, using a 2ml syringes. This was injected at 50mg/kg body weight (1.0ml/100g) to the experimental animals in groups II, IV and V intraperitonealy. All experimental animals were granted free access to normal feed and 10% sucrose water. Afterwards, 10% sucrose water was replaced with regular water and normal feed daily. After 72 hours, the rats were allowed to fast overnight, and then blood glucose was measured via a tail vein using one touch basic blood glucose monitoring system (glucose fine test strip) to evaluate blood glucose concentration. A rise in blood glucose was a positive confirmation for diabetes.

Plant sample collection and preparation

C. afer leaves were harvested along a garden at Tinapa road Calabar, Cross River State. These were identified and authenticated by a Plant taxonomist in the Department of Plant and Ecological Studies, University of Calabar, Calabar Nigeria. A voucher sample was deposited at the University of Calabar Herbarium with reference number Bot/Herb/UCC/305. The fresh leaf samples were rinsed in clean water thoroughly to remove debris, and dried at 30 – 400C using a mammert oven model (FM; C50.0270). Then, it was allowed to cool and crushed into powdered form using a manual blender model – (FNO4) (quakercity mild Philadelphia. Pa. USA) and packaged separately ready for analysis.

Extraction procedure

About 200g of the grinded *C. afer* leaf samples was accurately weighed using a weighing balance (AE – Adam model) and soaked in 1500mls of distilled water for 24 hours and was subsequently filtered using a Whatman no. 1 filter paper. The solution was concentrated into a syrupy residue at 400C -500C using a thermostatic water bath model – (F.Nr; L508.0271). The extract was weighed and stored in a well closed container and kept in a refrigerator at 40C to protect from light and moisture till used.

Administration of C. afer

C. afer was administered orally at a dosage of 800mg/kg body weight daily to rats in Group III (C. afer only) and Group V (*C. afer* and Diabetic treated group). *C. afer* was administered orally using an oral gavage. After administration of extract for two weeks, the animals were sacrificed and parameters analyzed.

Administration of metformin

Metformin was obtained from a standard pharmacy shop located at Etta Agbor, Calabar, Cross River state. Five hundred milligrams (500mg) of Metformin was grinded and dissolved in 25ml distilled water to constitute a stock concentration of 20mg/ml and was administered at a dose of 200mg/kg of body weight to the animal using oral gavage to group IV experimental animals only. After administration for two weeks, experimental animals were sacrificed and parameters analyzed.

Determination of food and water intake

Water intake was measured using calibrated feeding bottle with stainless steel nozzles. The daily water intake was obtained by subtracting the volume of water remaining at the end of 24 hours of feeding from the initial amount in the feeding bottle at the start of the day. The food intake was measured by weighing the amount of food left in the container after 24 hours and subtracting it from the initial amount of food at start of the day's feeding. The food containers were medium sized stainless steel plates to avoid spillage of food.

Liver function test

Blood was collected by cardiac puncture of the anaesthetized (chloroform-treated) animals. Blood samples from each rat were collected using syringes and needles and separated into samples bottles and allowed to stand for 30 minutes for clotting to take place. These were then centrifuged at 3000g for 10 minutes and the serum extracted into fresh test tubes and stored in a refrigerator for analysis of Alkaline phosphatase (ALP), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST).

Measurement of alkaline phosphatase, alanine and aspartate transferase

While measurement of alkaline phosphatase was achieved by the optimized standard method recommended by Schumann *et al.*,[31] the measurement of AST and ALT activities in the serum were done using method described by the International Federation of Clinical Chemistry (IFCC) and Laboratory Medicine.

Gastric acid study/Collection and measurement of pepsin

Gastric acid secretion was measured in all groups of animals by Gastric Cannulation with Automated Fractional Collection described by Goel *et al*.[32] Gastric juice used for analysis of pepsin was collected using modern enzymatic assay according to method described by Kudryavtsev *et al*.[33] Proteolytic activity was assessed using a fluorometric casein digestion assay kit (Abcam, UK), according to the manufacturer's instructions.

Extraction of adherent mucus and gastric ulceration studies

Adherent mucus weight was determined by the method of Tan *et al*.[34] Ulceration was induced in the rats before rats were sacrificed, according to the method of Joshi *et al*.[35] by gastric instillation of acid-ethanol. Ulcer index was calculated as the percentage

of the gastric mucosal surface affected. Ulcer score was done according to the following grading system
.

Ulcer index for each group = Number of rats X number of group / Total number of rats in a group

Ulcer incidence (%) = Number of rats with ulcer X 100/ Total number of rats

Grading of ulcer points:
Grade 0 - No lesions
Grade 1 – Haemorrhagic lesions (less than 5mm)
Grade 2 – Haemorrhagic lesions (Greater than 5mm or small linear ulcers)
Grade 3 – Many small linear ulcers (Greater than 2mm or a single linear)
Grade 4 – Multiple linear ulcers of marked size

Determination of fluid and glucose absorption
Glucose and fluid absorption were evaluated according to the method described by Hall and Hall.[36] Four intestinal segments (1, Il, ll and IV) each 10cm long (2 From jejunum and 2 from ileum) were cut out for making of sacs.

Statistical analysis
All results were presented as mean, standard error of mean (SEM). One way analysis of variance (ANOVA) was used to compare more than two means followed by Bonferroni's multiple comparison test to test for significant differences between groups. p<0.05 was considered statistically significant. Computer software (SPSS and Excel Analyzer) was used for the analysis.

Results and discussion
Table 1 shows the fasting blood glucose concentrations in the control, DM, *C. afer*, DM + MTF and DM + *C. afer* was 3.94 + 0.37, 4.34 + 0.28, 4.12+0.29, 4.26 + 0.28 and 4.32 +0.31 respectively. The result showed there was no significant difference in fasting blood glucose concentration among the groups before induction of diabetes, as presented in figure 1. Diabetes was induced in experimental animals after two weeks of acclimatization, using streptozotocin. The fasting blood glucose concentration in the control, DM, *C. afer*, DM + MTF and DM + *C. afer* was found to be 4.16 + 0.26, 9.46 + 0.18, 4.18 + 0.23, 9.08 + 0.69 and 9.18 + 0.57 respectively (Table 2 and figure 2). The result showed that DM, DM + *C. afer* and DM + MTF group was significantly higher (P<0.05) when compared to the control group while the *C. afer* group was significantly lower (P<0.05) when compared to the diabetic group. Lastly, DM + MTF and DM + *C. afer* group was significantly (P<0.05) higher when compared to the *C. afer* group. The blood glucose concentration after treatment of diabetes with the reference (metformin) and *C. afer* were 4.08±0.33, 9.82±0.76, 5.62±0.21,4.78±0.32 and 5.28±0.36 for control, DM, *C. afer*, DM + MTF and DM+*C. afer* groups respectively, (Table 3 and figure 3). There was a significant increase in fasting blood glucose concentration when the DM untreated group was compared to the control, while the *C. afer*, DM + MTF and DM+*C. afer* were significantly reduced compared to DM untreated group.
This result could be attributed to the extract ability to restore the destroyed pancreatic cells and enhance glucose utilization. Study by Boison *et al*.[9] attests to the fact that *C. afer* acts as an antidiabetic agent via biochemical mechanisms including restitution of pancreatic β-cell function, amelioration of insulin resistance by sensitizing receptors, inhibition of liver gluconeogenesis, enhanced glucose absorption, and

Table 1: Comparison of Fasting Blood Glucose Concentration in the Control, DM, *C. afer*, DM + MTF and DM + *C. afer* Groups.

Groups	Mean/SEM	Level of Significance P value (p>0.05)
Control	3.94 ± 0.37	
DM	4.34 ± 0.28	No significance
C. afer	4.12±0.29	No significance
DM + MTF	4.26 ± 0.28	No significance
DM + *C. afer*	4.32 ±0.31	No significance

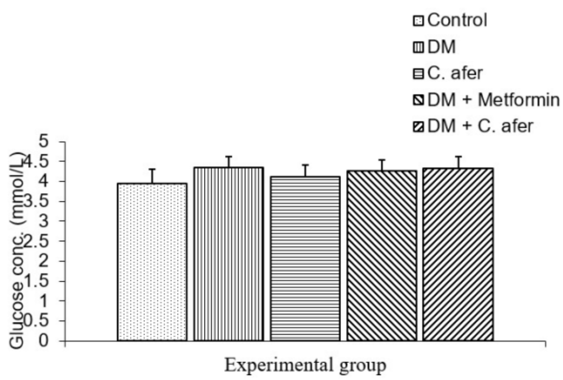

FIG 1: Glucose concentration before induction of diabetes in the different experimental groups.

Values are expressed as mean +SEM, n=7.
No significant differences among groups (p>0.05)

Table 2: Comparison of Blood Glucose Concentration after induction of Diabetes in Control, DM, *C. afer*, DM+MTF and DM+ *C. afer* groups.

Groups	Mean/SEM	Level of Significance P value (p<0.05)
Control	4.16 ± 0.26	
DM	9.46 ± 0.18*	* vs control
C. afer	4.18 ± 0.23a	a vs DM
DM + MTF	9.08 ± 0.69*b	*b vs control and C. afer
DM + *C. afer*	9.18 ± 0.57*b	*b vs control and C. afer

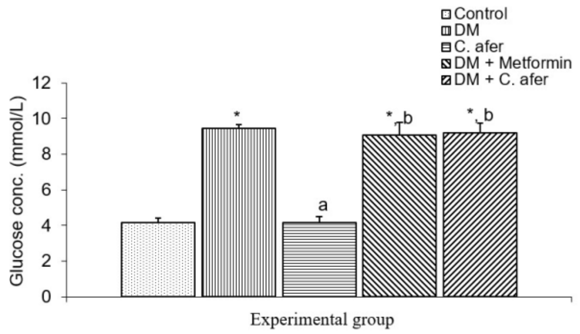

FIG.2: Glucose concentration after induction of diabetes in the different experimental groups.

Values are expressed as mean +SEM, n =7.
* = p<0.05 vs control
a = p<0.05 vs DM
b = p<0.05 vs *C. afer*

Table 3: Glucose Concentration after Treatment of Diabetes in the Different Experimental Groups.

Groups	Mean/SEM	Level of Significance P value (p<0.05)
Control	4.08±0.33	
DM	9.82±0.76*	* vs control
C. afer	5.62±0.21,4a	a vs DM
DM + MTF	4.78±0.32a	a vs DM
DM + *C. afer*	5.28±0.36a	a vs DM

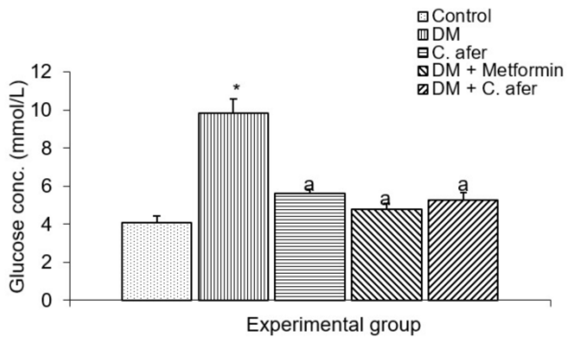

FIG. 3:Glucose concentration after treatment of diabetes in the different experimental groups.

Values are expressed as mean +SEM, n = 7.
* = p<0.05 vs control
a = p<0.05 vs DM

inhibition of glucose-6-phosphatase, α-amylase, and α-glucosidase activities. The stem and roots of *C. afer* are reported to contain several bioactive compounds with diosgenin and aferosides A, B, and C named as the most likely compounds responsible for the antidiabetic properties of *C. afer*.[10,11] Hence, the reason for observed reduced blood glucose concentration seen after treatments in the *C. afer* group.

The mean and SEM glucose uptake values were 1.07±0.17, 0.09±0.06, 1.8±0.15, 1.08±0.18 and1.9±0.22 for control, DM, *C. afer*, DM + MTF, DM + *C. afer* groups respectively (Table 4). The result shows a significant decrease (p<0.05) in DM and DM + MTF group compared to control and a significant increase in *C. afer* and DM + *C. afer* group compared to control and DM group. DM + MTF group showed no significant difference compared to DM group. Absorption is dependent on the rate of change of basolateral spaces which in turn is determined by the connective tissues and solutes like sodium or glucose, which implies, if glucose absorption is disturbed, fluid absorption will be affected too. However, diabetics suffer from frequent thirst which leads to polydipsia (frequent drinking of water). This might explain the increased gut fluid uptake seen in the diabetic group, in addition, *C. afer* is rich in electrolytes such as potassium, sodium and chloride ions,[9] hence the composition of these electrolytes might have even contributed to increased water consumption, hence the significant increase observed in the *C. afer* and DM + *C. afer* groups compared to the control.

Glucose absorption was again significantly higher in *C. afer* and DM + *C. afer* group compared to the corresponding control. This signified intestinal glucose absorption was not affected by *C. afer.* The increased glucose absorption seen in these groups can be attributed to the high carbohydrate and electrolyte content of the plant, but rather the blood glucose concentration was kept in check by the *C. afer* since it has been shown to ameliorate insulin resistance by sensitizing receptors, inhibition of liver gluconeogenesis, enhanced glucose absorption from gut, and inhibition of G-6-Phosphatase, α-amylase, and α-glucosidase activities.[10]

The mean and SEM values for feed intake were 25.76±2.22, 29.15±3.21, 24.16±0.1.91, 18.19±0.1.77 and 23.94±2.05 for control, DM, *C. afer*, DM + MTF DM+*C. afer* groups respectively. The result showed a significant increase (p<0.05) in DM and decrease in DM+MTF group compared to control group. There

Table 4: Glucose transfer by small intestinal segments of control and experimental groups.

Groups	Glucose concentration Before incubation (mmol/gsac/30mins)		Glucose transfer after incubation		Glucose uptake
	Mucosal	Serosal	Mucosal	Serosal	Gut glucose uptake
Control	4.93±0.23	4.93±0.23	4.63±0.26	3.56±0.01	1.07±0.17
DM	5.09±0.19	5.09±0.19	4.52±0.32	4.43±0.03	0.09±0.06*
C. afer	5.08±0.17	5.08±0.17	4.29±0.21	2.49±0.03	1.8±0.15*a
DM+MTF	5.07±0.9	5.07±0.9	4.26±0.12	3.26±0.02	1.08±0.18*
DM+*C. afer*	5.01±0.6	5.01±0.6	5.35±0.17	3.45±0.03	1.9±0.22*a

Gut fluid transfer in the different experimental groups.
Values are expressed as mean ± SEM, n = 7
* p<0.05 vs control
a = p<0.05 vs DM

was also a significant decrease in *C. afer* and DM+*C. afer* group when compare to DM group, while DM+MTF group showed a significant decrease compare to *C. afer* group and DM + *C. afer* groups (Table 5 and figure 4). This result connotes the C. afer extract was responsible for observed reduce feed intake. *C. afer* is rich in a variety of phytochemicals which includes steroidal sapogenins, aferosides, dioscin, and paryphyllin C and flavonoid glycoside kaempferol-3-O-α-L-rhamnopyranoside and tannins. The phytochemical tannin has been reported to decrease feed intake and feed efficiency in experimental animals.[11] The hypothalamus acts as the control center for hunger and satiety. Part of the hypothalamus, the arcuate nucleus (or, in humans, the infundibular nucleus), allows entry through the blood-brain barrier of peripheral peptides and proteins that directly interact with its neurons. These include neurons that coexpress peptides that stimulate food intake and weight gain, specifically, neuropeptide-Y[12] and agouti-related peptide (AgRP), as well as those expressing pro-opiomelanocortin (POMC). Furthermore, *C. afer* is rich in macromolecules such as carbohydrate and crude proteins.[13] This probable mechanism might be the reason for reduced feed intake.[9]

The mean and SEM values for water intake were 3.240±0.09274, 6.078±0.09790, 2.540±0.06782, 5.180±0.1241 and 1.980±0.1281 for control, DM, *C. afer*, DM + MTF DM + *C. afer* groups respectively (Table 6 and figure 5). A similar pattern to feed intake was observed for water intake with the most reduced water intake seen in the *C. afer* and DM+ *C. afer* groups. Whilst, DM Untreated and DM+*C. afer* groups were inversely increased compared to the control group. This is not surprising for the DM untreated group, because diabetics often suffer from polydipsia due to dehydration caused by excretion of water (polyuria) which follows glucose excretion due to hyperglycemia.[14] With regards to the reference drug MTF, a significant reduction in feed intake observed is attributed to its ability to reduce energy consumption and weight gain in DM.[15] Hence, the likely reason for the reduction of weight seen in DM+MTF group.

Table 5: Feed intake in the Control and Experimental Groups

Groups	Mean/SEM	Level of Significance
Control	25.76±2.22	
DM	29.15±3.21*	* vs control
C. afer	24.16±0.1.91a	a vs DM
DM + MTF	18.19±0.1.77*b	*b vs control and *C. afer*
DM + *C. afer*	23.94±2.05ac	ac vs DM and DM+MTF

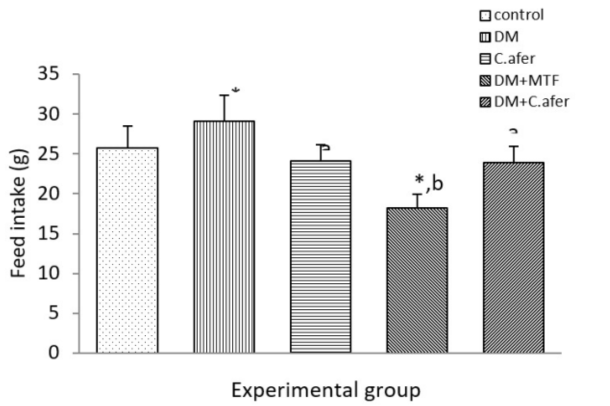

FIG. 4: Mean feed intake in control and different experimental group

Values are expressed as mean +SEM, n = 7.* = p<0.05 vs controla = p<0.05 vs DMb = p<0.05 vs *C. afer* c= p<0.05 vs DM+*C. afer*

However, a significant reduction of body weight changes seen in DM untreated (180±21.34, 186±20.3, 179±19.2, 183±26.3 and 187±17.9 for control, DM, *C. afer*, DM + MTF DM+*C. afer* groups respectively, (Table 7 and figure 6) had an inverse relationship with feed intake. Despite the high food intake which connotes polyphagia in DM,[16] the animals still had reduced body weight over time. This only depicts the wasting syndrome associated with diabetes mellitus due to abnormal metabolism of macromolecules and malabsorption. Contrary, the *C. afer* and DM + *C. afer* group had a significant increase in body weight despite a reduction in food intake compared to the DM untreated group. The reason for this might be the ability of *C. afer* to abate the diabetic effect by its insulin restorative effect, because in the absence of insulin as seen in type II DM or in receptors insensitivity to insulin, there is abnormal metabolism of macromolecules, where by energy production is switched from carbohydrate to non-carbohydrate as proteins (muscles) and fats precursors.[17]

A review on *C. afer* by Boison *et al*.[9] clearly attests to the fact that *C. afer* acts as an antidiabetic agent via biochemical mechanisms including restitution of pancreatic β-cell function, amelioration of insulin resistance by sensitizing receptors, inhibition of liver gluconeogenesis, enhanced glucose absorption, and inhibition of G-6-Pase, α-amylase, and α-glucosidase activities,[9] and insulin is a major hormone responsible for the catabolism and anabolism of major macromolecules,[18] thus, the probable reason for the restorative effect of body weight seen in DM + *C. afer* group. In addition, *C. afer* is a rich source of both micro and macronutrients such as carbohydrates, crude proteins and lipids, ergo; cumulatively these

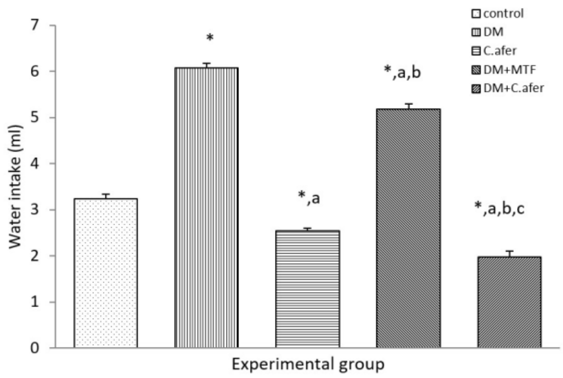

FIG. 5: Mean water intake in control and different experimental group

Values are expressed as mean +SEM, n =7. * = p<0.05 vs controla = p<0.05 vs DMb = p<0.05 vs *C. afer* c= p<0.05 vs DM+*C. afer*

Table 7: Initial and Final Body Weight Changes in the Control and Experimental Groups

Groups	Initial	Final	Level of Significance P value (p<0.05)
Control	180±21.34	222±24.1	
DM	186±20.3	153 ±22.1*	* vs control
C. afer	179±19.2	210 ±23.5a	a vs DM
DM + MTF	183±26.3	145 ± 13.4*b	*b vs control and C. afer
DM + *C. afer*	187±17.9	201 ± 21.5ac	ac vs DM and DM+MTF

Table 6: Water intake in the control and experimental groups.

Groups	Mean/SEM	Level of Significance P value (p<0.05)
Control	3.240±0.09274	
DM	6.078±0.09790*	* vs control
C. afer	2.540±0.06782*a	*a vs control and DM
DM + MTF	1.980±0.1281*ab	*ab vs control, DM and C. afer
DM + *C. afer*	1.980±0.1281*abc	*abc vs control, DM, C. afer and DM+MTF

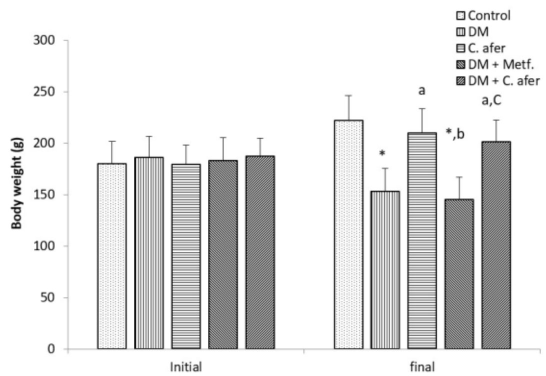

FIG. 6: Initial and final body weight in control and different experimental group

Values are expressed as mean +SEM, n =7. * = p<0.05 vs controla = p<0.05 vs DMb = p<0.05 vs *C. afer* c= p<0.05 vs DM+*C. afer*

might have probably resulted to the gain in body weight seen in this group. The result is in consonance with a study by Ezejiofor *et al*.,[19] which demonstrated no change in body weight of animals treated with aqueous leaves of *C. afer* in non-diabetic rats as seen in the *C. afer* group.

The Basal gastric acid in the control, DM, *C. afer*, DM + MTF, DM + *C. afer* were 36.20 ± 0.10, 16.80 ± 0.04, 19.20 ± 0.07, 29.40 ± 0.07 and 29.00 ± 0.05 respectively, (Table 8, figure 7 and 8). The results show clearly that the basal acid secretion in all the experimental groups was significantly lower (P<0.005) when compared to their corresponding control group. Again, the *C. afer*, DM + MTF & DM + *C. afer* were significantly lower (P<0.05) when compared to diabetic group. However, DM + MTF and DM + *C. afer* was significantly higher (P<0.05) compared to the *C. afer* group.

Figures 7 and 8 illustrates histamine injection in the control, DM, *C. afer*, DM + MTF, DM + *C. afer* groups were 50.20 ± 0.09, 85.60 ± 0.40, 31.20 ± 0.23, 70.40 ± 0.28, 53.00 ± 0.39 respectively. The results showed that there was a significantly higher difference in the DM, DM + MTF when compared to the control, while the *C. afer* group showed no significant lower difference when compared to the control. Furthermore, the *C. afer*, DM + MTF and DM + *C. afer* was significantly (P<0.05) higher when compared to the diabetic group, while the DM + *C. afer* and DM + MTF was significantly higher than the *C. afer* group. Lastly, the *C. afer* group also showed a significant reduction when compared to the DM +

MTF group. Figure 7 and 8 shows the histamine-stimulated acid output when challenged using cimetidine, a H2 Blocker. The result for the control, DM, *C. afer*, DM + MTF, DM + *C. afer* were 32.80 ± 0.11, 29.40 ± 0.41, 31.60 ± 0.15, 33.80 ± 0.15 and 36.28 ± 0.85 respectively, (Table 8). The results showed there was no significant difference in the experimental groups when compared with their control and amongst the groups. The normal stomach mucosa maintains a balance between protective and aggressive factors. Some of the main aggressive factors are gastric acid, abnormal motility, pepsin, bile salts, use of alcohol and no steroidal anti-inflammatory drugs (NSAID), as well as infection with microbes (Helicobacter pylori and others). On the other hand, mucus secretion, bicarbonate production, gastroprotective prostaglandin synthesis and normal tissue microcirculation protects against ulcer formation. Although in most cases the etiology of ulcer is unknown yet, it is generally accepted that gastric ulcers are multifactorial and develop when aggressive factors (endogenous, exogenous and/or infectious agents) overcome mucosal defense mechanisms.[16,19]

In this study, there was a decrease in basal acid secretion in all the experimental groups compared to the control groups and an increased acid secretion in *C. afer*, DM+MTF and DM+*C. afer* group compared to the DM untreated groups. A study by Owu *et al.*[20] suggests diabetes induces cellular and functional changes in the glandular stomach especially in the parietal cells such as the decrease in the number of mitochondria accompanied by reduction in H+-K+-ATPase and canaliculi in parietal cells which may explain the reduced acid secretion observed in diabetes.[21] Also, notable changes that are often observed in patients with chronic diabetes mellitus include decreased gastric acid secretion.[22] Nonetheless, *C. afer* also caused a significantly reduced basal acid output in *C. afer* and DM+ *C. afer* groups. This finding is in line with a study by Khattab & Aljehany,[23] which demonstrated a reduction in gastric acid and pH of rats treated with *C. afer* extract.

When histamine, an H2 agonist was administered, histamine stimulated gastric acid output significantly in all experimental groups except the group treated *C. afer* only which was decreased significantly compared to the control and other experimental groups. The reason for this is quite unclear, but it might suggest the extracts from *C. afer* leaves interfered with the gastric acid secretory histaminergic pathway, with a probable histaminergic receptor blocker. Evaluation of the adherent mucus was significantly increased in *C. afer* and DM+*C. afer* groups, with *C. afer* showing the peak increase in mucus secretion. Evaluation of phytochemical constituents of *C. afer* shows it is rich in flavonoids[24] and flavonoids possesses anti-ulcer effects which includes functions such as anti-acid secretion, and increasing gastric mucus and bicarbonate secretion. In addition, flavonoids boost mucosal cytoprotective, antioxidative, anti-inflammatory, and antibacterial defenses against peptic ulcer. Usually, one type of flavonoid can exhibit anti-ulcer roles through multiple mechanisms.[25] Ergo, the most likely reason why *C. afer* increased mucus secretion in both *C. afer* and DM+*C. afer* groups.

Evaluation of pepsin concentration had a result similar to that of mucus secretion for *C. afer* group and DM+*C. afer* group. Again, this is implicative on the flavonoid phytochemical content of the plant, because flavonoid have been shown to reduce pepsin

Table 8: Comparison of aqueous extract of *C. afer* leaves and gastric acid secretion, mucus output, pepsin concentration and gastric ulceration in control and experimental groups

Groups	Basal gastric acid	Histamine	Cimetidine	Level of Significance P value (p<0.05)
Control	36.20±0.10	50.20±0.09	32.80±0.11	
DM	16.80±0.04*	85.60±0.40*	29.40±0.41	*vs control
C. afer	19.20±0.07*a	31.20±0.23*a	31.60±0.15	*a vs control and DM
DM + MTF	29.40±0.07*ab	70.40±0.28*ab	33.80±0.15	*ab vs control, DM and *C. afer*
DM−*C. afer*	29.00±0.05*a	53.00±0.39*abc	36.28±0.85	*abc vs control, DM, *C. afer* and DM−MTF

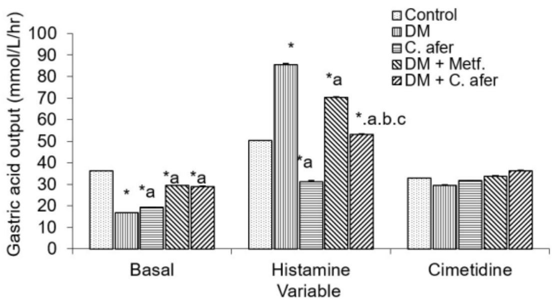

FIG. 7: Effect of treatment with metformin and *C. afer* on basal, histamine and cimetidine induced gastric secretion.

Values are expressed as mean +SEM, n = 7.

* = p<0.05 vs control

a = p<0.05 vs DM

b = p<0.05 vs *C. afer*

c = p<0.05 vs DM + Metformin.

secretion.[25] Interestingly, ulcer scores revealed a similar pattern showing a protective effect of the gastric mucosa of *C. afer* group and DM+*C. afer* groups. This is because the defensive factors such as increased mucus secretion, reduced pepsin secretion and reduced basal acid secretion prevented against ulcer formation, since these are all defensive factors, hence prevented ulcer formation as seen in *C. afer* and DM+*C. afer* groups.

The mean and SEM values for ulcer scores were 7.0±0.2, 11.1±0.7, 5.1±0.4, 6.9±0.4 and 4.4±0.3 for control, DM, *C. afer*, DM + MTF, DM + *C. afer* groups respectively, (Table 9 and figure 9). The result shows a significant increase ($p<0.05$) in DM group compared to control and a significant decrease in DM + *C. afer* group compared to control group. *C. afer*, DM + MTF and DM + *C. afer* group showed a significant decrease compared to DM group, DM + *C. afer* also decreased compared to DM + MTF group.

The mean aspartate aminotransferase (AST) for the control, DM, *C. afer*, DM + MTF and DM + *C. afer* were 118.40±1.47, 179.20±86, 147.40±1.96, 79.80±1.16 and 104.40±1.72 respectively, (Table 10 and figure 10). The DM+*C. afer* group showed a

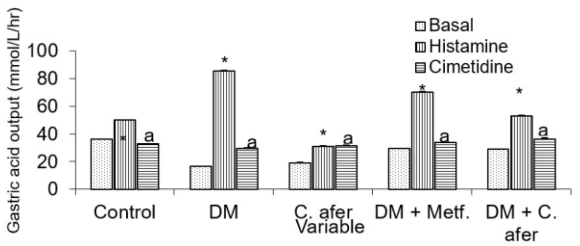

FIG. 8: Effect of histamine and cimetidine on basal gastric acid output in the different experimental groups.

Values are expressed as mean +SEM, n = 7.
* = $p<0.05$ vs basal
a = $p<0.05$ vs histamine

Table 9: Comparison of aqueous extract of *C. afer* leaves on gastric ulceration in control and experimental groups

Groups	Mean/SEM	Level of Significance P value ($p<0.05$)
Control	7.0±0.2	
DM	11.1±0.7*	* vs control
C. afer	5.1±0.4a	a vs DM
DM + MTF	6.9±0.4a	a vs DM
DM + *C. afer*	4.4±0.3*ac	*ac vs control, DM and DM+MTF

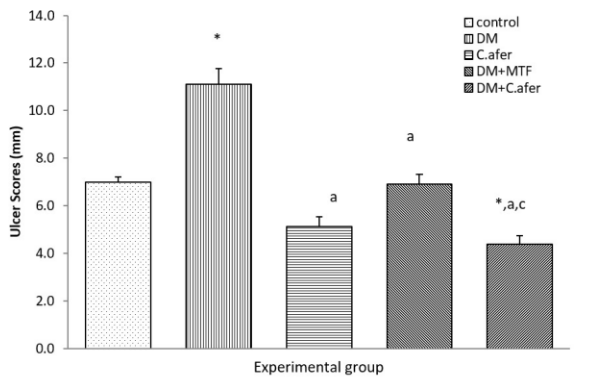

FIG. 9: ulcer scores in control and different experimental groups

Values are expressed as mean +SEM, n = 7. * = $p<0.05$ vs control a = $p<0.05$ vs DM b = $p<0.05$ vs *C. afer* c= $p<0.05$ vs DM+*C. afer*

significant increase ($p<0.05$) in AST concentration when compared to the control. Whereas, the DM + MTF and DM + *C. afer* group showed a significant reduction ($p<0.05$) in AST concentration when compared to control group. However, there was a significant reduction ($p<0.05$) in DM + *C. afer* and DM + MTF when compared to the diabetic group and *C. afer* only group, while the DM + MTF group had a significant increase when compared to the DM group. In addition, the DM + MTF group was significantly reduced ($p<0.05$) when compared to DM + *C. afer* group. AST showed a significant increase in both test groups (DM and *C. afer* groups), while there was a significant reduction in DM + MTF and DM + *C. afer* group. Although AST is a less specific biomarkers of liver dysfunction compared to ALT[26] owing to its multi-location in different tissues and organs such as the heart, kidneys and muscles.[27] The increase observed in DM group could be attributed to free radicals as diabetics have been shown to have increased free radical production which can attack virtually all the cells in the body and cause leakage of this multi located enzyme. In addition, the increase in AST in the *C. afer* group could be attributed to the toxic effect of the alkaloid which is a phytochemical present in *C. afer*. Since this extract was administered by oral gavage, there might have been a toxic effect to the liver via the portal circulation as this is the first contact of the extract with the liver. However, the DM + MTF and DM + *C. afer* group had a reduced concentration of AST. This could be attributed to the treatment during the administration of metformin and this probably is due to the amelioration of oxidative stress caused by metformin and *C. afer*. Contrary, there was an increase in AST in the diabetic and *C. afer* treated groups only, and the reason for this is not clearly understood.

By contrast, ALT is the most specific marker of liver injury because it is located largely in the liver.[28] This study recorded a significant increase in the diabetic group and *C. afer*, while the DM + MTF group was significantly reduced. The mean Alanine aminotransferase concentration in the control, DM, *C. afer*, DM + MTF and DM + *C. afer* were 74.80 ± 1.20, 104.60 ± 133, 89.20 ± 1.66, 60.00 ± 1.14 and 70.00 ± 0.95 respectively, (Table 10 and figure 11). The DM and *C. afer* group showed a significant increase (p<0.05) when compared to the control. Contrary, the DM + MTF showed a significant reduction (p<0.05) when compared to control. Furthermore, there was a significant reduction when the DM + MTF and DM + *C. afer* group is compared to the DM group and the *C. afer* group. While the *C. afer* group showed a significant increase when compared to the DM group as well. However, there was a significant increase was observed when the DM + *C. afer* was compared to the DM + MTF group. In addition, the DM + *C. afer* group was significantly reduced (p<0.05) when compared to DM + MTF group. The reduction seen in the metformin group could be as a result of reduced oxidative stress by metformin which prevented hepatocellular damage and leakage of ALT into the blood.

The mean Alkaline phosphatase concentration in the control, DM, *C. afer*, DM + MTF and DM + *C. afer* were 163.20 ± 3.87, 178.20 ± 1.16, 164.60 ± 1.08, 124.20 ± 1.53 and 154.00 ± 1.30 respectively, (Table 10 and figure 12). The DM + *C. afer* and DM + MTF groups showed a significant reduction (p<0.05) when compared to the control. Contrary to this, the DM group was significantly higher (p<0.05) when compared to control. However, there was a significant reduction in DM + MTF and DM + *C. afer* group is compared to the DM group and the *C. afer* group. While there was a significant increase observed when the DM + MTF was compared to the DM + MTF group. The significant reduction in all experimental groups when compared to diabetic group strongly suggests effectiveness of phytochemicals present in the plant extract. A similar finding was earlier reported by Boison *et al.*[9] who observed the same trend in rodents treated with *C. afer*. However, the increase observed in ALP concentration in the diabetic untreated group could be attributed to other extra hepatic tissues, since ALP is commonly found in bone disease or renal hypo perfusion and this is a common symptom of diabetes.[29]

Table 10: Comparison of aqueous extract of *C. afer* leaves on liver enzymes in control and experimental groups

Groups	AST	ALT	ALP	Level of Significance P value (p<0.05)
Control	118.40±1.47	74.80±1.20	163.20±3.87	
DM	179.20±86*	104.60±133*	178.20±1.16*	*vs control
C. afer	147.40±1.96*a	89.20±1.66*a	164.60±1.08a	*a vs control and DM
DM+MTF	79.80±1.16*ab	60.00±1.14*ab	124.20±1.53*ab	*ab vs control. DM and C. afer
DM+*C. afer*	104.40±1.72*abc	70.00±0.95abc	154.00±1.30*abc	*abc vs control, DM. C. afer and DM+MFT

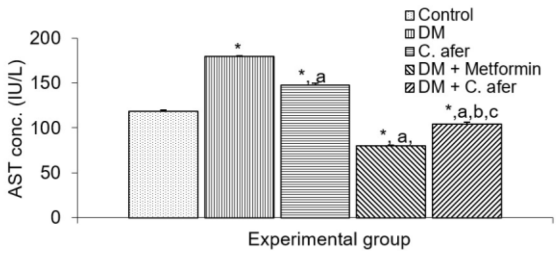

FIG. 10: Aspartate aminotransferase concentration before induction of diabetes in the different experimental groups.

Values are expressed as mean +SEM, n = 7.
* = p<0.05 vs control
a = p<0.05 vs DM
b = p<0.05 vs *C. afer*
c = p<0.05 vs DM + Metformin

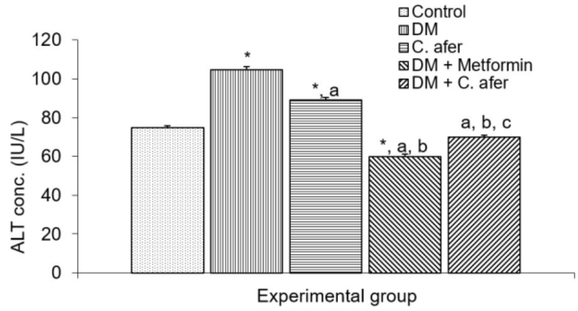

FIG. 11: Alanine aminotransferase concentration after induction of diabetes in the different experimental groups.

Values are expressed as mean +SEM, n = 7.
* = p<0.05 vs control
a = p<0.05 vs DM
b = p<0.05 vs *C. afer*
c = p<0.05 vs DM + Metformin

This increase is also in agreement with the study of Rapuri *et al*.[30] who observed similar increase in diabetic patients.

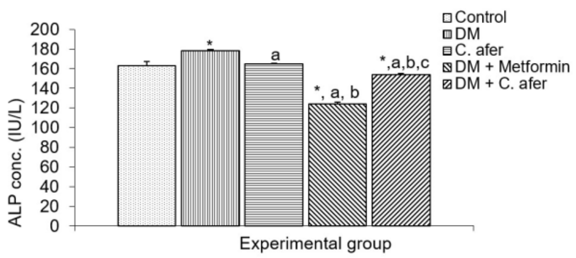

FIG. 12: Alkaline phosphatase concentration after treatment of diabetes in the different experimental groups.

Values are expressed as mean +SEM, n = 7.
* = p<0.05 vs control
a = p<0.05 vs DM
b = p<0.05 vs *C. afer*
c = p<0.05 vs DM + Metformin

Conclusion

Since *C. afer* significantly reduced blood glucose concentration, water intake, feed intake, body weight, basal acid secretion, mucus secretion, blood glucose, pepsin concentration, ulcer scores, AST, ALT, ALP, gut glucose transfer, but increased favorably, mucus secretion and gut glucose transfer. Thus, consumption or use of aqueous extract of *C. afer* at a dosage of 800mg/kg body weight maybe beneficial in managing hyperglycemia and treating gastrointestinal symptoms associated with diabetes mellitus

Competing interest

The authors declare no competing financial interest.

Authors' contribution

APU was responsible for the overall supervision of the manuscript; GCG wrote the protocol and analyzed the data; OAI and EDO supervised data collection and wrote the introduction and discussion; UJM and APU wrote the methodology; while EDO and GCG wrote the results and interpreted the data. All authors have read and agreed to the final manuscript.

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