

Gastrointestinal and Hepatobiliary Effects of Aqueous Extract of *Costus afer* Leaves in Diabetic Wistar Rats

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ABSTRACT

Aim: Diabetes mellitus (DM) remains a global health challenge, with escalating incidence and prevalence across developed and developing nations, infecting approximately 537 million in 2021 and is projected to increase to 783 million by 2045. Gastroparesis, dyspepsia, and peptic ulceration are common diabetes-associated complications within the gastrointestinal system. This study aimed to investigate the gastrointestinal and hepatobiliary effects of aqueous extract of *C. afer* leaves in diabetic Wistar rats.

Materials and Methods: The leaves of *C. afer* were collected, identified, dried, ground into powder, and extracted with distilled water. The aqueous extract was administered orally to Wistar rats at 800mg/kg body weight. Thirty female rats were randomized into five groups: one receiving only *C. afer*, one receiving metformin (200mg/kg), and others as controls. The study involved monitoring food and water intake, collecting biliary secretion, and measuring bile electrolytes, bilirubin, and cholesterol levels. Fecal lipids, glucose, and protein content were also analyzed. Statistical analysis was done using ANOVA, with $p < 0.05$ considered significant.

Results: The study revealed significant differences across treatment groups. Pepsin concentrations were reduced in *C. afer* (0.24 ± 0.10), DM+MTF (0.29 ± 0.02), and DM+*C. afer* (0.17 ± 0.03) compared to control (0.42 ± 0.02). Ulcer scores were higher in DM (11.1 ± 0.7) and lower in DM+*C. afer* (4.4 ± 0.3). Bicarbonate levels decreased in DM (19.8 ± 0.66) and *C. afer* (18.2 ± 0.80) compared to control (23.4 ± 0.87). Chloride and sodium ions showed significant variations. Potassium increased in *C. afer* (3.38 ± 0.11), DM+MTF (5.22 ± 0.10), and DM+*C. afer* (3.92 ± 0.09). Total bilirubin was higher in DM+MTF (2.48 ± 0.13) and DM+*C. afer* (2.42 ± 0.13).

Conclusion: *C. afer* demonstrated antidiabetic effects by reducing blood glucose, enhancing glucose absorption, modulating gut motility, and influencing biliary electrolyte balance, though it may pose a risk for electrolyte imbalances and cholelithiasis.

Keywords: Bioactivity; Natural products; Medicinal plants; *C. afer*

INTRODUCTION

Diabetes mellitus (DM) remains a global health challenge, with escalating incidence and prevalence across developed and developing nations. According to the International Diabetes Federation, the global number of adults with diabetes reached approximately 537 million in 2021 and is projected to increase to 783 million by 2045. DM adversely impacts multiple organ systems,

including the gastrointestinal (GI) tract and hepatobiliary axis [1]. Within the GI system, gastroparesis, dyspepsia, and peptic ulceration are common diabetes-associated complications [2]. These conditions are mediated by effects such as autonomic neuropathy, oxidative stress, and impaired mucosal defense. Animal models have demonstrated that hyperglycemia leads to delayed gastric emptying and enzyme alterations—most notably pepsin

oversecretion, which can exacerbate mucosal injury [3].

Hepatobiliary dysfunctions, including non-alcoholic fatty liver disease (NAFLD), elevated liver enzymes (ALT, AST, ALP), cholestatic changes, and dyslipidemia, are frequently observed in individuals with DM [4,5]. Disruptions in bilirubin metabolism and cholesterol levels often accompany hepatic stress. Addressing both GI and liver dysfunctions in conjunction with glycemic control could offer more comprehensive therapeutic benefits. While first-line antidiabetic drugs such as metformin and insulin sensitizers effectively lower blood glucose, they often lack organ-specific benefits and may carry side effects like GI upset or lactic acidosis [6]. Such side effects are uncommon with treatments associated with plant extracts.

Costus afer Ker Gawl. (Costaceae), commonly referred to in English as “African spiral ginger,” is a perennial herb native to West Africa. Traditional medicine across Nigeria attributes antidiabetic, antimicrobial, antiulcer, anti-inflammatory, and hepatoprotective properties to *C. afer*. Leaf decoctions and stem extracts are frequently used to treat fever, hyperglycemia, gastrointestinal disturbances, and liver disease [7,8]. Phytochemical profiling confirms that *C. afer* leaves contain a diverse array of bioactive compounds such as flavonoids, terpenoids, glycosides, tannins, alkaloids, and phenolic acids. These have associated biological activities such as antioxidant, anti-inflammatory, enzyme inhibition, gastric cytoprotection, and lipid modulation [9,10]. Goodies et al. [7] demonstrated significant antihyperglycemic effect of aqueous *C. afer* extract in alloxan-induced diabetic Wistar rats, with no major hepatotoxicity or acute toxicity for up to 28 days at doses up to 400 mg/kg. The extract reduced fasting blood glucose

comparably to glibenclamide without altering liver enzyme biomarkers (AST, ALT, ALP).

Similarly, Ezejiofor et al. [11] conducted a series of studies using aqueous leaf and stem extracts of *C. afer* in alloxan-induced Wistar rats. They consistently reported dose-dependent reduction in blood glucose, restoration of pancreatic beta-cell morphology, and normalization of liver function parameters (ALT, AST, ALP, total bilirubin) and kidney markers (urea, creatinine), with histopathologic regeneration in hepatocytes, renal tubules, and pancreas. Okwuanga et al. [8] also investigated the ability of *C. afer* stem juice to counteract ethanol-induced liver toxicity in female Wistar rats, finding significant reductions in ALT, AST, ALP and improved liver histology, suggesting potent hepatoprotective and antioxidant effects in non-diabetic hepatic injury models. These studies predominantly concentrate on glycemic control, pancreatic regeneration, hepatic enzyme normalization, and antioxidant activity. However, data remain scarce on the impact of *C. afer* on gastric physiology, specifically pepsin secretion and ulcer formation, and on detailed hepatobiliary markers like conjugated/unconjugated bilirubin and cholesterol in diabetic conditions. This study therefore aims to investigate the gastrointestinal and hepatobiliary effects of aqueous extract of *C. afer* leaves in diabetic Wistar rats.

MATERIAL AND METHODS

Plant materials/sample collection

The leaves of *C. afer* were meticulously collected from a garden situated along Tinapa Road in Calabar, Cross River State. The specimen was identified and authenticated by Dr. Effa Effa A. from the Department of Plant and Ecological Studies at the University of Calabar, Nigeria. A voucher specimen has been deposited in the University of Calabar

Herbarium, designated with the reference number Bot/Herb/UCC/305.

Sample preparation

The freshly obtained leaf samples were thoroughly rinsed in distilled water to eliminate any extraneous debris. Subsequently, the samples were dried at a temperature range of 30 – 40°C utilizing a man-made Mamert oven model (FM; C50.0270). Following this, samples were allowed to cool and then ground into a fine powder using a manual blender model (FNO4) (Quaker City Mild, Philadelphia, PA, USA) and were packaged separately in preparation for analysis.

Extraction procedure

Using a weighing balance (Model: AE - Adam), 200 grams of the ground *C. afer* was weighed and immersed in 1500 ml of distilled water for a duration of 24 hours, after which it was filtered through Whatman no. 1 filter paper. The resultant solution was concentrated into a syrupy residue at a temperature of 40°C - 50°C using a thermostatic water bath (F.Nr; L508.0271). The extract was subsequently

weighed and stored in a tightly sealed container, maintained in a refrigerator at 4°C to shield it from light and moisture until required for use.

Choice of experimental animals and maintenance

A total of thirty (30) female rats, each weighing within the range of 160 to 200 grams, were procured from the animal facility of the Faculty of Basic Medical Sciences, College of Medicine, University of Calabar, located in Cross River State, Nigeria. The rats were allowed to undergo a period of acclimatization lasting fourteen days in specially designed cages that ensured adequate ventilation at a controlled ambient temperature of 28 to 30°C degrees Celsius, under a 12-hour light and 12-hour dark photoperiod. During this acclimatization phase, the rats were provided with a diet consisting of rat pellets and had unrestricted access to clean water. The animals were subsequently randomized into five distinct groups, each comprising six rats (Table 1).

Table 1: Experimental design and animal grouping

No.	GROUPS	NO. OF RATS	FEEDING REGIMEN
1.	Control group	6	Standard rat pellets + drinking water
2.	Diabetic group	6	Standard rat pellets + drinking water + Streptozotocin
3.	<i>C. afer</i> group	6	Standard rat pellets + drinking water + <i>C. afer</i>
4.	Diabetes + Metformin group	6	Standard rat pellets + drinking water + Streptozotocin + Metformin
5.	Diabetes + <i>C. afer</i> group	6	Standard rat pellets + drinking water + Streptozotocin + <i>C. afer</i>

Ethical approval

Ethical Approval was obtained from the animal ethical committee of the Faculty of Basic Medical Science, University of Calabar, Calabar, Nigeria. The guidelines of the ethical committee were applied to all procedures used for the experimental animals.

Collection and preparation of plant samples

The leaves of *C. afer* were collected from a garden situated along Tinapa Road in Calabar, Cross River State. These were identified and authenticated by Dr. Effa Effa A. of the Department of Plant and Ecological Studies at the University of Calabar, Calabar, Nigeria. A voucher specimen was deposited in the University of Calabar Herbarium, assigned the reference number Bot/Herb/UCC/305. The freshly harvested leaf samples were thoroughly rinsed with clean water to eliminate any debris and subsequently dried at temperatures ranging from 30 to 40°C utilizing a Mammert oven model (FM; C50.0270). Following the drying process, samples were allowed to cool and were then ground into a fine powder using a manual blender model (FNO4) (Quaker City Mill, Philadelphia, PA, USA) and stored in separate containers in preparation for subsequent analysis.

Extraction procedure

Approximately 200 g of the finely ground *C. afer* leaf samples were weighed using an analytical balance (AE – Adam model) and soaked in 1500ml of distilled water for a duration of 24 hours. This solution was then filtered using Whatman No. 1 filter paper. The resultant filtrate was concentrated into a syrupy residue at a temperature range of 40 to 50°C in a thermostatic water bath model (F.Nr; L508.0271). The extract was weighed, placed in a tightly sealed container, and refrigerated at 4°C to safeguard against exposure to light and moisture until required for use.

Induction of diabetes mellitus

Following the period of acclimatization, and prior to the administration of the injection, 4ml of Streptozotocin (STZ) were dissolved in 50 mM sodium citrate buffer (pH 4.5) to achieve a final concentration of 4 mg/ml, utilizing a 2ml syringe. Streptozotocin was then administered at a dosage of 50 mg/kg body weight (1.0 ml/100 g) to the experimental animals in groups II, IV, and V via the intraperitoneal route.

All animals receiving Streptozotocin were provided with unrestricted access to standard feed and 10% sucrose water; subsequently, the 10% sucrose water was replaced with regular water and standard feed on a daily basis. After a period of 72 hours, the rats were subjected to a fasting period overnight, after which blood glucose levels were assessed via a tail vein puncture using a One Touch Basic blood glucose monitoring system (glucose fine test strip) to determine blood glucose concentration. An elevation in blood glucose levels served as a definitive confirmation of the diabetic state.

Administration of *C. afer*

C. afer was administered orally at a dosage of 800mg/kg body weight (similar dosage adopted by literature review) 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 Betz pharmacy at Etta Agbor, Calabar, Cross River state. 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 4 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 difference was the amount consumed for 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.

Administration of *C. afer*

The administration of *C. afer* was conducted orally at a dosage of 800mg/kg of body weight, which aligns with the dosages established in the existing literature, on a daily basis for the rats in Group III (*C. afer* only) and Group V (*C. afer* and diabetic treatment group). *C. afer* was delivered orally utilizing an oral gavage technique. Following a two-week period of extract administration, the subjects were sacrificed, and various parameters were subsequently analyzed.

Administration of metformin

Metformin was procured from Betz Pharmacy located in Etta Agbor, Calabar, Cross River State. Metformin (500mg) was dissolved in 25ml of distilled water to formulate a stock concentration of 20mg/ml, which was administered at a dosage of 200mg/kg of body weight to the experimental animals in Group 4 via oral gavage. After a two-week administration period, the experimental animals were sacrificed for the analysis of various parameters.

Determination of food and water intake

Water intake was quantified using a calibrated feeding bottle equipped with stainless steel nozzles. The daily water intake was calculated by deducting the volume of water remaining at the conclusion of a 24-hour feeding period from the initial volume present in the feeding bottle at the commencement of the day. The resultant difference represented the quantity consumed throughout the day. The measurement of food intake was executed by weighing the residual amount of food in the container after a 24-hour period and subtracting this from the initial quantity of food available at the outset of the feeding day. The food containers employed were medium-sized stainless steel plates to mitigate any potential spillage of food.

Collection of biliary secretion

Biliary secretion was collected by the method of Vickers *et al.* [12]. The rats were starved for 15 hours prior to the time of experiment. The animals were weighed and anaesthetized by intraperitoneal administration of 25% urethane (0.6ml/100g body weight), and were quickly pinned to a dissecting board for a tracheostomy performed to clear the airways for easy breathing. The abdomen was opened along the linea alba to prevent bleeding. The common bile duct was exposed by deflecting the liver lobes aside. The common bile duct was then cannulated with a portex cannula (0.5 mm in diameter) after a small incision was made. The cannula was held in place with two pieces of thread tied round the bile duct. The bile content was collected at 3 hours interval for each group studied.

Determination of biliary electrolytes

Sodium and potassium ions in bile were determined using Flame photometer (Model 4100, Petracourt Ltd England). The bile was sprayed into a nonluminous gas flame which became coloured by the characteristic emission of metallic ions in the sample. The

wavelength of the metals 598nm and 767nm for sodium and potassium respectively were selected by a light prism system and allowed to fall on a photosensitive detection system. The amount of light emitted is dependent on the concentration of the metallic ion present by comparing the amount of light emitted from the sample with that from the standard solution. The amount of sodium and potassium were determined.

Determination of bicarbonate in bile

Plasma Carbon dioxide (CO₂) was measured by the modified method of Forrester *et al.* [13] Principle: Phosphoenol pyruvate carboxylase (PEPC) catalyzes the reaction between phosphoenol pyruvate and carbon dioxide (bicarbonate) to form oxaloacetate and phosphate ions with simultaneous oxidation of an equimolar amount of reduced NADH (Nicotinamide adenosine dinucleotide) to NAD. The reaction is catalyzed by malate dehydrogenase (MDH). This results in a decrease in absorbance at 340nm that is directly proportional to CO₂ concentration in the sample.

Determination of biliary chloride

The method of Kolkoff *et al.* [14] was used. Two mls of buffer solution were placed in a conical flask and 0.2ml of bile was added and mixed. Four drops of diphenyl carbazene indicator were added. This was titrated with mercuric nitrate from a 2ml micro pipette. The end point was indicated by a violet colour. The standard solution (0.2ml) was added to 2.0ml buffer solution and indicator added and similarly titrated.

Determination of biliary bilirubin

This was done according to method of Sherlock [15]. Total bilirubin is the sum of conjugated and unconjugated bilirubin. Conjugated bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form blue colour complex. The total bilirubin was

determined in the presence of caffeine which released albumin bound bilirubin on reacting with diazotized sulphanilic acid. The colour produced was read spectrophotometrically at 530-560nm.

Extraction of cholesterol from bile secretion

Cholesterol was determined using the method of Allain *et al.* [16]. Free cholesterol and cholesterol ester were released from lipoproteins by the action of surfactant. Then both existing free cholesterol and cholesterol released from its ester by cholesteryl hydrolase were oxidized by cholesterol oxidase. The resulting hydrogen peroxide reacts in the presence of peroxidase with the chromogen system 4-amine antipyrines phenol to produce a red colour compound. The red quinone formed was proportional to the amount of cholesterol present.

Moisture loss

The weighed fecal materials were dried in an oven at 90°C for 6 hours. The difference in weight after drying was the moisture loss. Fecal moisture content of 80% was considered to indicate the clinical diarrhea [17].

Determination of total fecal lipids

Total frozen dry fecal matter from each rat was ground with mortar and pestle into a homogenous mixture. Fecal lipid was extracted using a modification of the method of Folch *et al.* [18]. A small amount of water (0.5ml) was added to 0.5g of frozen dried faeces, methanol (5ml) followed by 10ml chloroform were added to each tube which was shaken and heated in a hot water bath (60°C) for 1 hour [19]. The solution was transferred to a second test tube. The remaining solid residues were re-extracted with methanol and chloroform and heated at 60° C for 1 hour in a water bath, Potassium chloride was added to the combined filtrate.

The test tube was shaken and centrifuged at 265 x g for 5 minutes to obtain 2 distinct layers. The bottom layer containing the purified lipid was transferred to a pre-weighed test tube and dried under nitrogen until solid lipid residue remained. The tube was dried over night to ensure complete drying of the sample. The weight of the total lipid was determined by the difference in weight.

Determination of fecal electrolytes

One gram (1g) of fecal material was collected from the different groups. The fecal material was put in 50ml barrel. Fifteen ml of distilled water was then added and left to homogenize for 30 minutes. The homogenate was filtered using Whatman number 1 filter paper. The supernatant was then transferred into plastic containers and frozen at -15° C. The electrolytes including Na⁺, K⁺ and Cl⁻ were determined by the principle of ion selective electrode.

Determination of fecal glucose and protein

A gram of fecal material was collected from all the groups, weighed and put into a small 50ml barrel. Fifteen ml of distilled water was then added and left to homogenize for 30 minutes. The homogenate was filtered using Whatman number 1 filter paper. The supernatant was then transferred into plastic containers and refrigerated and used for analysis of fecal protein and glucose. Biuret reagents (3g of copper sulphate) were dissolved with 12g of potassium sodium tartrate (KNaC₄H₄O₆·4H₂O) in approximately 1 litre of water. It was then transferred qualitatively to a 2 litre volumetric flask. Carbonate free sodium hydroxide was added into the solution to make a final base concentration of 3% (W/V). It was then stored in a polyethylene bottle. One percent (1%) NaCl was prepared by dissolving 10g of NaCl in distilled water and made up to 1 litre.

Fecal glucose estimation

The method of God-Pap [20] was used to determine fecal glucose estimation. In the presence of glucose oxidase, glucose was oxidized to glucuronic acid and hydrogen peroxide. The hydrogen peroxide reacts in the presence of peroxidase with phenol and 4-amino phenozone to form a quinone dye (pink) whose colour intensity was in proportion to the glucose concentration in the sample.

Statistical analysis

All results were displayed as the mean ± standard error of the mean (SEM). A one-way analysis of variance (ANOVA) was employed to compare the means of more than two groups, followed by Bonferroni's multiple comparison test to ascertain statistically significant differences among groups. The chi-square test was utilized to evaluate the significance of differences between means of percentages. A p-value of less than 0.05 (p<0.05) was deemed statistically significant. Computational software (SPSS and Excel Analyzer) was employed for the analytical procedures.

RESULTS

Comparison of aqueous extract of *C. afer* leaves on pepsin concentration in control and experimental groups

Table 2 and figure 1 show the mean concentrations for pepsin concentration were 0.42±0.02, 0.59± 0.05, 0.24 ±0.1, 0.29± 0.02 and 0.17 ± 0.03 for control, DM+*C. afer*, DM +MTF and DM+ *C. afer* respectively. There was a significant reduction in *C. afer*, DM +MTF and DM+*C. afer* group when compared to the control and DM untreated group. Inversely, the DM untreated group was significantly increased compared to other treated groups and control. However, the DM+MTF and DM+*C. afer* groups were significantly increased and decreased respectively when compared to the *C. afer*

group. While, the DM+C. *afer* group was significantly reduced compared to the DM+ C. *afer* group was significantly reduced compared to the DM +MTF treated group.

Table 2: Comparison of aqueous extract of *C. afer* leaves on pepsin concentration in control and experimental groups.

Groups	Mean/SEM	Level of Significance (P value <0.05)
Control	0.42±0.02	
DM	0.59± 0.05*	*vs control
<i>C. afer</i>	0.24 ±0.1*a	*a vs control and DM
DM + MTF	0.29± 0.02*b	*b vs control and C. <i>afer</i>
DM + <i>C. afer</i>	0.17 ± 0.03*abc	*abc vs control, DM, C. <i>afer</i> and DM+MTF

Comparison of aqueous extract of *C. afer* leaves on gastric ulceration in control and experimental 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 3

and figure 2). 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.

Table 3: Comparison of aqueous extract of *C. afer* leaves on gastric ulceration in control and experimental groups.

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

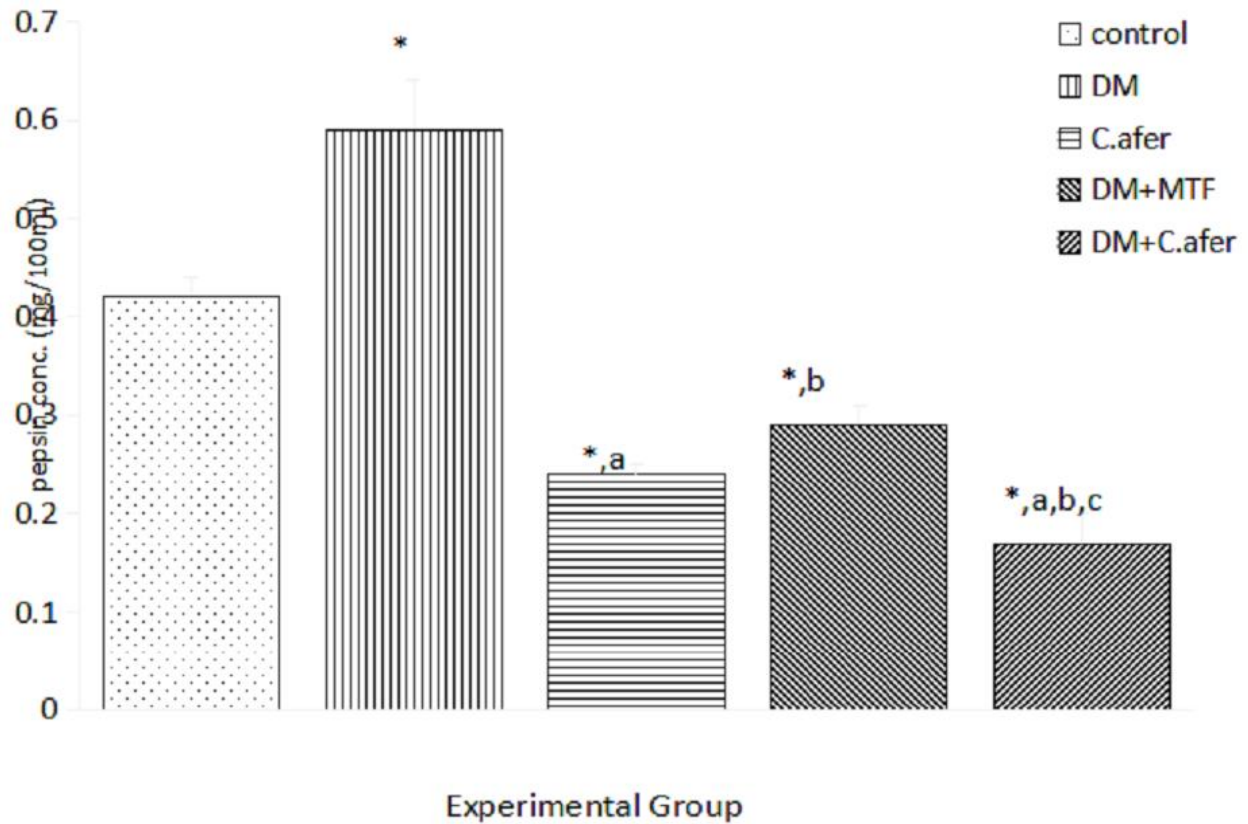


FIG. 1: Pepsin concentration in the different experimental groups.

Values are expressed as mean $\pm$ 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*

Table 4: Comparison of aqueous extract of *C. afer* leaves on rate of biliary secretion in control and experimental groups.

Groups	Mean/SEM	Level of Significance
Control	0.20 $\pm$ 0.01	-
DM	0.24 $\pm$ 0.8	No significance
<i>C. afer</i>	0.27 $\pm$ 0.6	No significance
DM + MTF	0.25 $\pm$ 0.02	No significance
DM + <i>C. afer</i>	0.35 $\pm$ 0.03	No significance

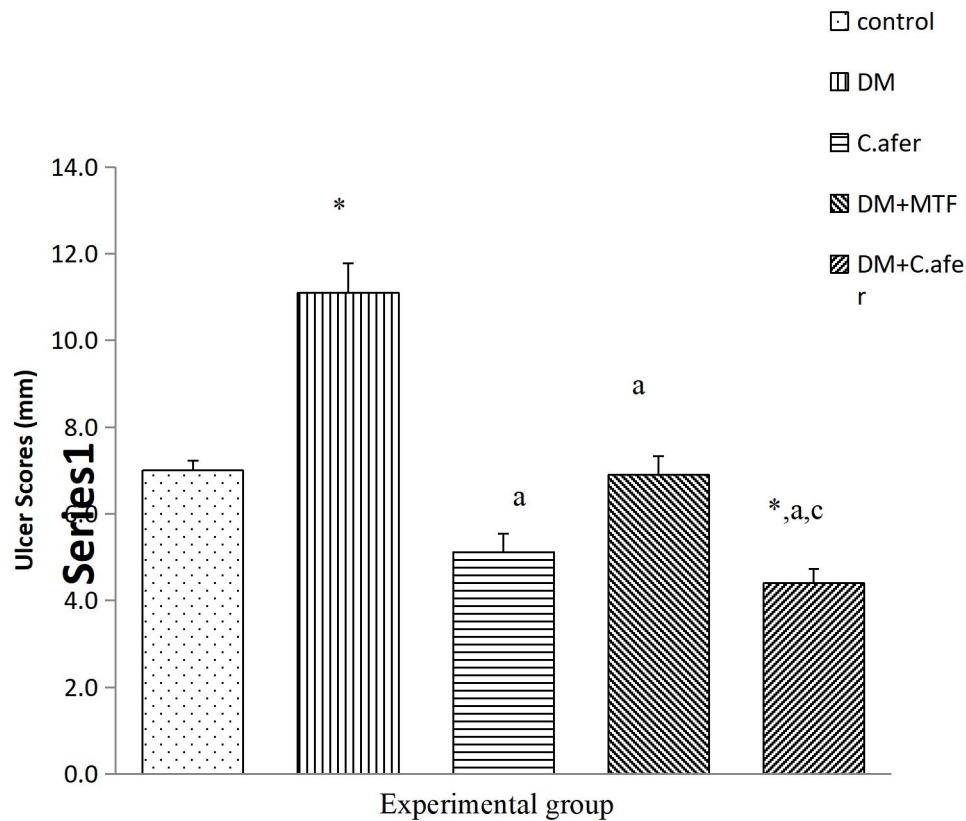


FIG. 2: Ulcer scores in control and different experimental groups

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

Comparison of aqueous extract of *C. afer* leaves on rate of biliary secretion in control and experimental groups.

The mean biliary flow rate in the control, DM, *C. afer*, DM + MTF and DM + *C. afer* group

was 0.20 ± 0.01 , 0.24 ± 0.8 , 0.27 ± 0.6 , 0.25 ± 0.02 , and 0.35 ± 0.03 respectively (Table 4 and figure 3). There was no significant difference in the mean biliary flow rate among the group.

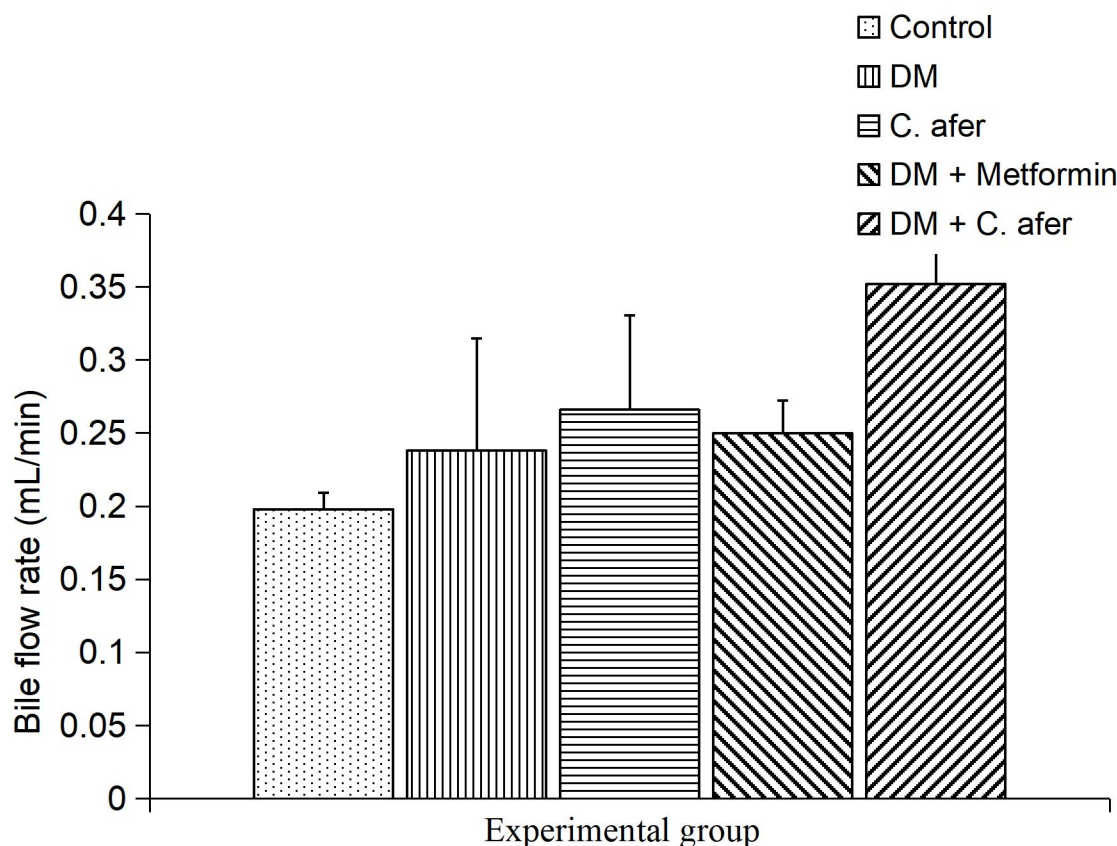


FIG. 3: Biliary flow rate in the different experimental groups.

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

No significant differences among groups

Comparison of Biliary electrolyte concentration in control, DM, *C. afer*, DM+MTF and DM+ *C. afer* groups.

Analysis of the mean Bicarbonate ion concentration was 23.40 ± 0.87 , 19.80 ± 0.66 , 18.20 ± 0.80 , 20.00 ± 0.89 and 21.40 ± 0.81 for control, DM, *C. afer*, DM + MTF, DM + *C. afer* group respectively (Table 5 and figure 4). There was a significant reduction ($p < 0.005$) in the DM and *C. afer* group when compared to the control group. However, the DM + MTF and DM + *C. afer* group showed no significant difference when compared to other groups.

The mean chloride ion concentration in the control, DM, *C. afer*, DM + MTF and DM + *C. afer* were 90.80 ± 1.6 , 94.40 ± 1.50 , 112.60 ± 2.01 , 105.20 ± 0.80 and 94.40 ± 93 respectively (Table 5 and figure 5). There was a significant increase ($p < 0.05$) when the *C. afer*, and DM + MTF group was compared to the corresponding control. Also, there was a significant increase ($p < 0.05$) of chloride ions when the *C. afer* and DM + MTF group was compared with the diabetic group. Contrary, the DM + MTF and DM + *C. afer* group were significantly reduced ($p < 0.005$) when compared to the *C. afer* only group. However, the DM + *C. afer* group was significantly

reduced ($p<0.05$) when compared to the DM + MTF group.

The mean biliary sodium ion concentration for the control, DM, *C. afer*, DM + MTF and DM + *C. afer* were 131.40 ± 0.87 , 127.60 ± 0.87 , 134.60 ± 0.121 , 133.00 ± 1.00 and 126.20 ± 0.66 respectively (Table 5 and figure 6). There was a significant reduction ($p<0.05$) in the DM and DM + MTF, DM + *C. afer* group when compared to the control. Contrary, A significant increase ($p<0.05$) was observed in the DM + MTF when compared to the control. However, a significant increase ($p<0.05$) was shown when the *C. afer* group and DM + MTF group were compared to the DM group. On the other hand, there is a significant reduction ($p<0.05$) when the DM + MTF and DM + *C. afer* group were compared to the *C. afer* group. Lastly, a significantly

reduction was observed when DM + *C. afer* was compared to the DM + MTF group.

The mean potassium ion concentrations in the control, *C. afer*, DM + MTF and DM + *C. afer* were 3.26 ± 0.05 , 3.38 ± 0.11 , 5.22 ± 0.10 , 4.56 ± 0.08 and 3.92 ± 0.09 respectively (Table 4 and figure. 7). There was a significant increase ($p<0.05$) in the *C. afer*, DM + MTF, DM + *C. afer* group when compared to the control groups and again to the diabetic groups. in addition, the DM + MTF and DM + *C. afer* group showed a significant increase ($p<0.005$) when compared to the *C. afer* only group. However, the DM + *C. afer* group was significantly reduced ($p<0.05$) when compared to the DM + MTF group.

Table 5: Comparison of Biliary electrolyte concentration in control, DM, *C. afer*, DM+MTF and DM+ *C. afer* groups.

Groups	HCO ₃ ⁻	Cl ⁻	Na ⁺	K ⁺	Level of Significance P value ($p<0.05$)
Control	23.40 ± 0.87	90.80 ± 1.6	131.40 ± 0.87	3.26 ± 0.05	-
DM	$19.80 \pm 0.66^*$	94.40 ± 1.50	$127.60 \pm 0.87^*$	3.38 ± 0.11	* vs control
<i>C. afer</i>	$18.20 \pm 0.80^*$	$112.60 \pm 2.01^{*a}$	$134.60 \pm 0.121a$	$5.22 \pm 0.10^{*a}$	*a vs control and DM
DM+MTF	20.00 ± 0.89	$105.20 \pm 0.80^{*ab}$	$133.00 \pm 1.00^{*ab}$	$4.56 \pm 0.08^{*ab}$	*ab vs control, DM and <i>C. afer</i>
DM+C. <i>afer</i>	21.40 ± 0.81	$94.40 \pm 93bc$	$126.20 \pm 0.66^{*bc}$	$3.92 \pm 0.09^{*abc}$	*abc vs control, DM, <i>C. afer</i> and DM+MTF

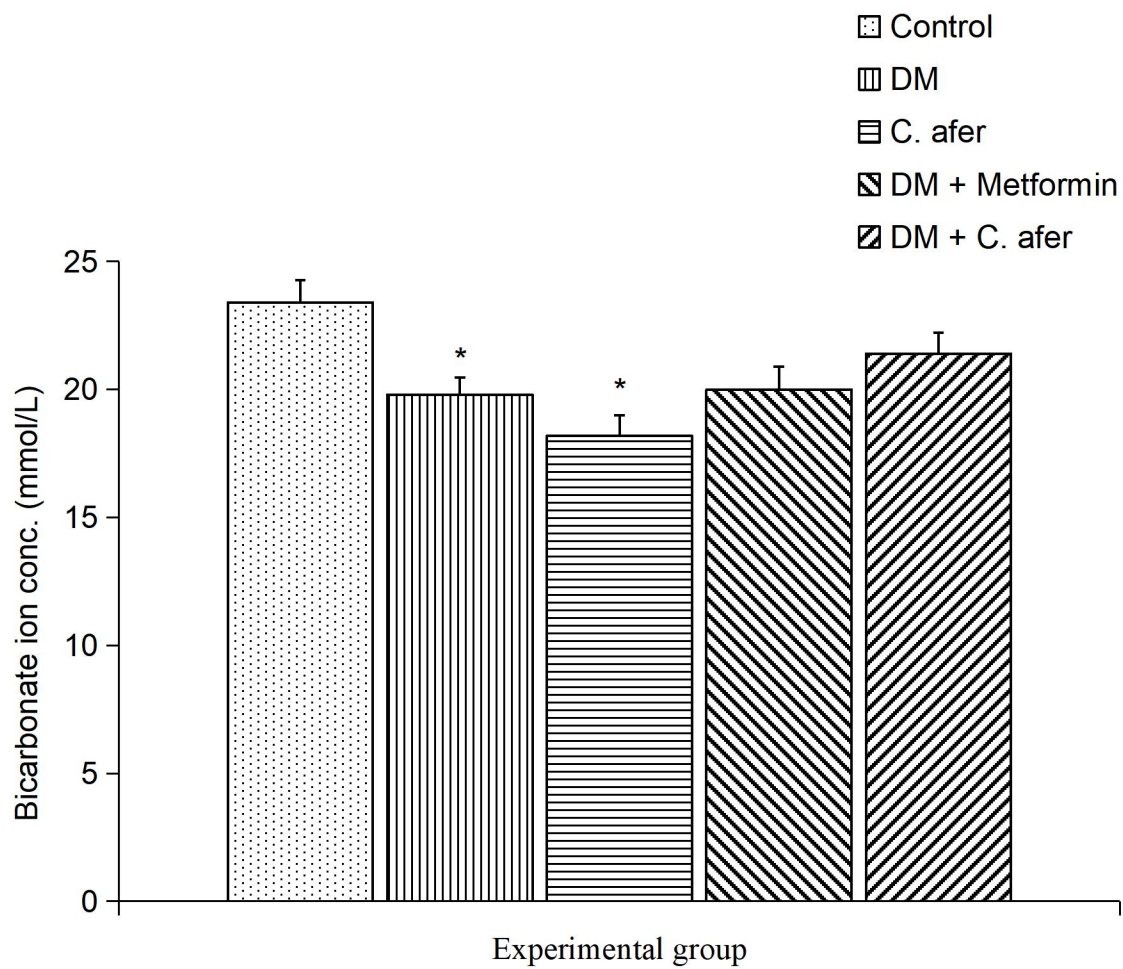


FIG. 4 : Bicarbonate ion concentration in the different experimental groups.

Values are expressed as mean +SEM, n = 7

* = $p < 0.05$ vs control

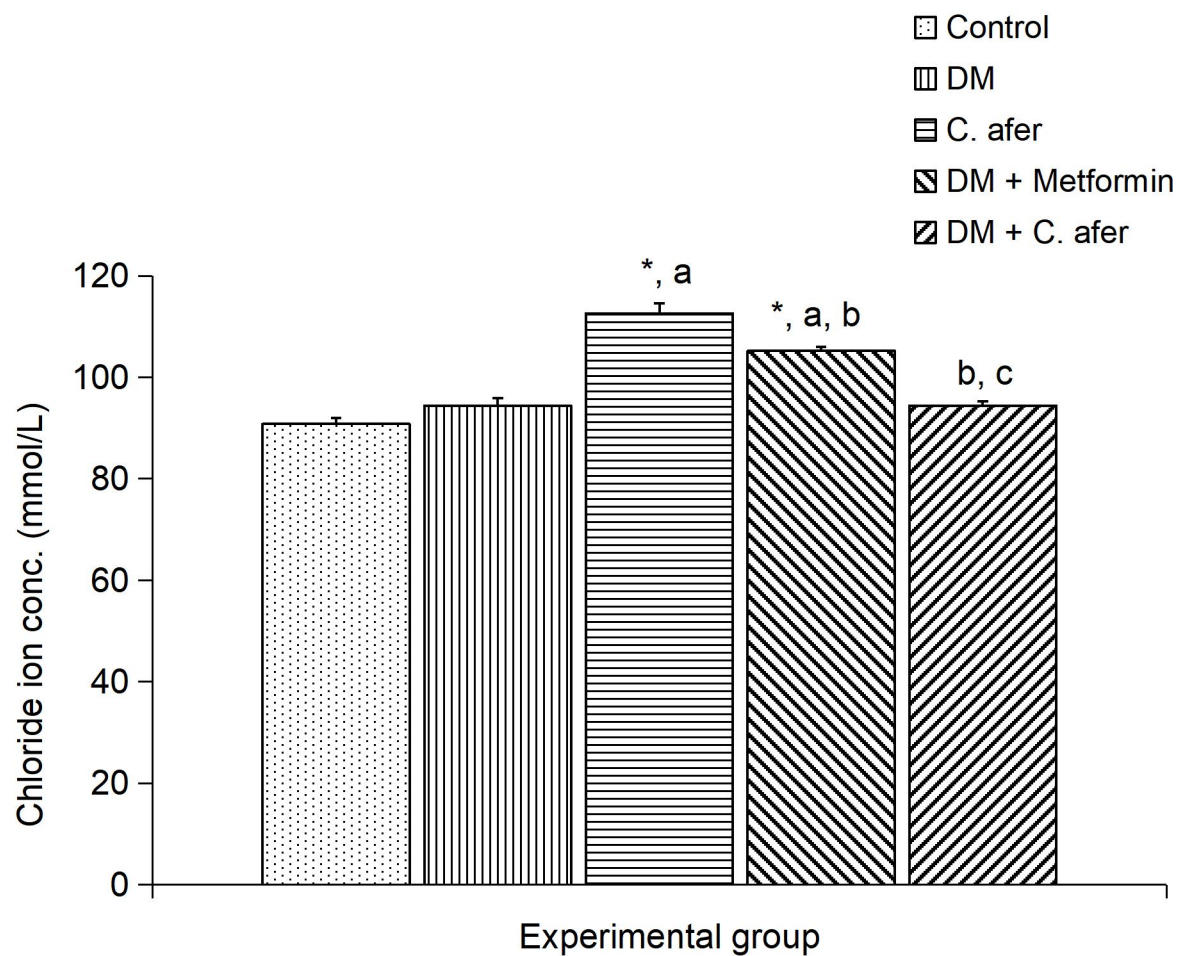


FIG. 5: Chloride ion concentration in the different experimental groups.

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

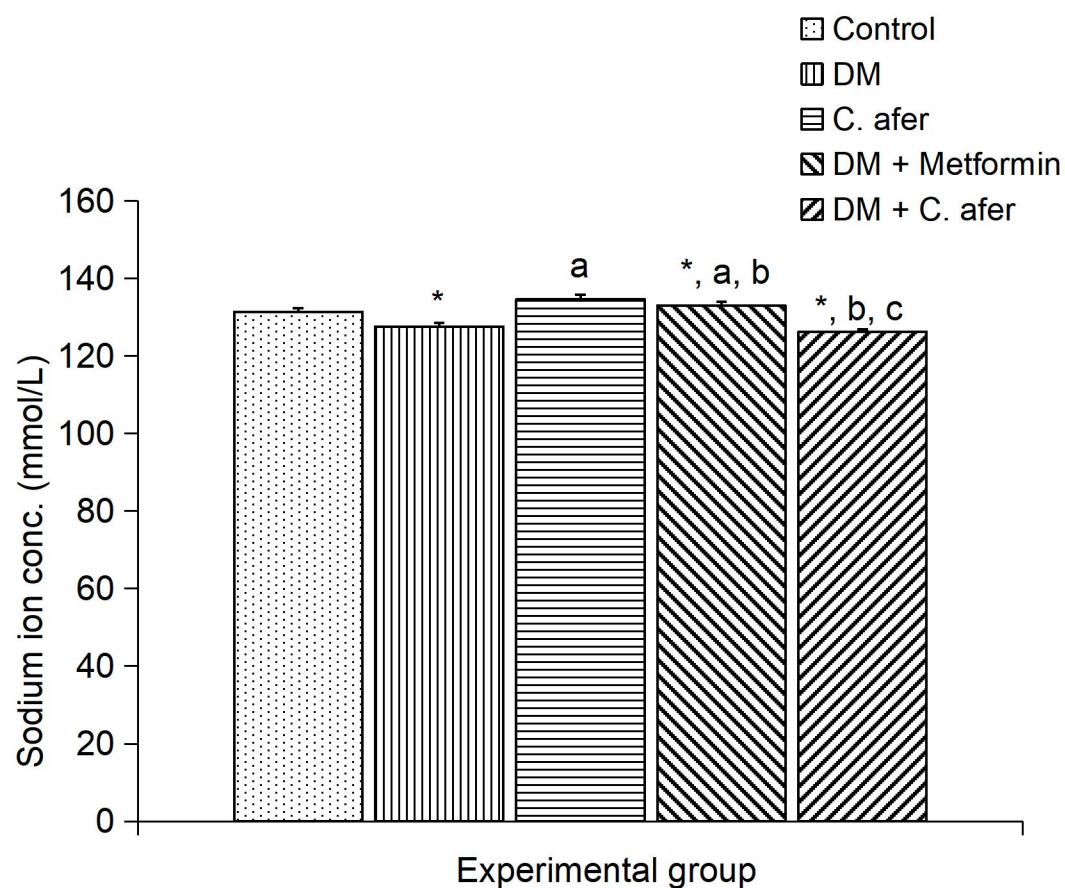


FIG. 6: Sodium ion concentration in the different experimental groups.

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

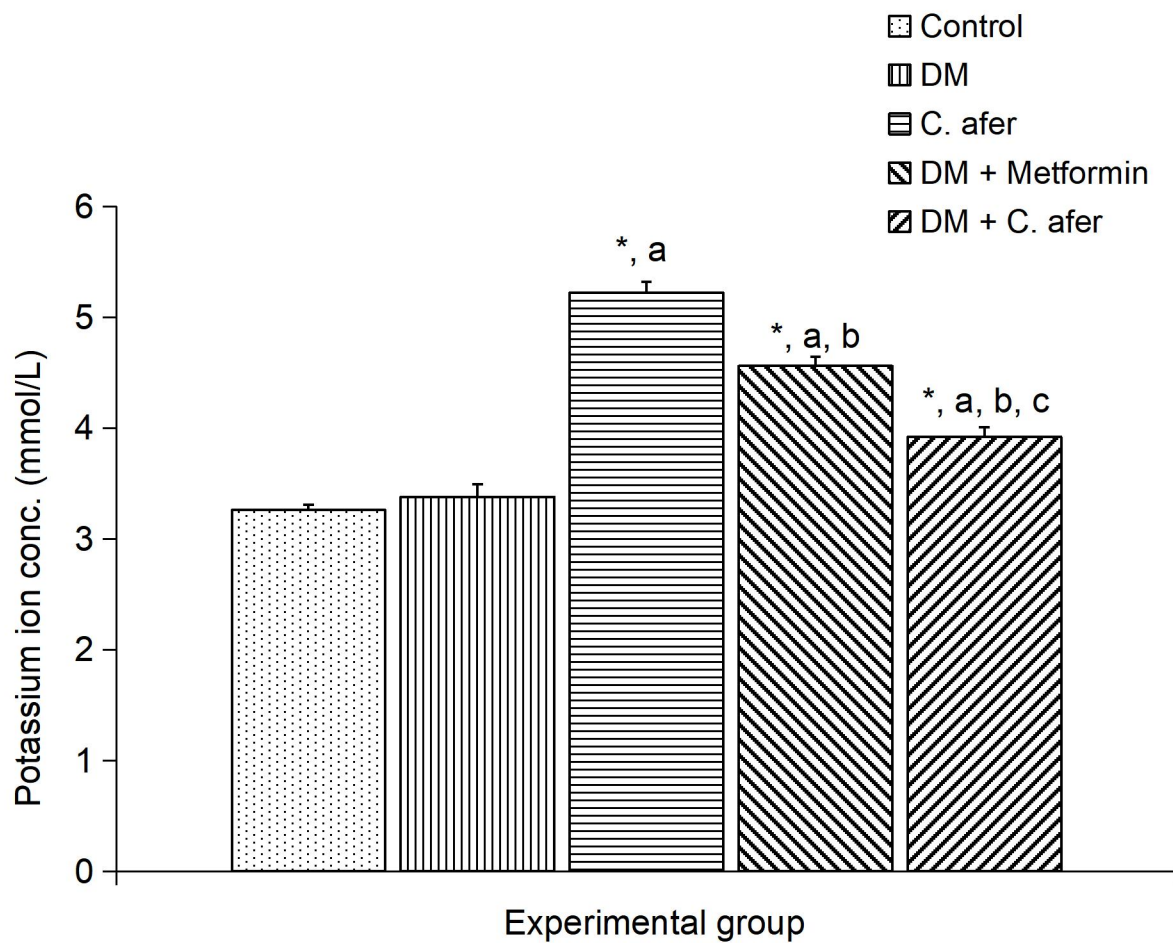


FIG. 7: Potassium ion concentration in the different experimental groups.

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

Comparison of aqueous extract of *C. afer* leaves on total, conjugated, unconjugated bilirubin and cholesterol levels in control and experimental groups.

The mean values of total bilirubin for control, DM *C. afer*, DM +MTF and DM +*C. afer* were 1.74 ± 0.06 , 2.12 ± 0.06 , 1.46 ± 0.10 , 2.48 ± 0.13 and 2.42 ± 0.13 respectively (Table 6 and figure 8). There was a significant increase in DM+*C. afer* DM+MTF groups when compared to the control group and *C. afer* group only, while the *C. afer* group was significantly increase in DM+MTF group when compared to the DM+*C. afer* group only, while the *C. afer* group was significantly reduced compared to the DM untreated group. The mean values for conjugated bilirubin in the control, DM, *C. afer*, DM +MTF and DM +*C. afer* groups

were 1.26 ± 0.13 , 1.64 ± 0.12 , 1.32 ± 0.11 , 1.68 ± 0.12 and 1.24 ± 0.09 . There was no significant difference when compared amongst groups and corresponding control.

The mean values for the concentration of unconjugated bilirubin in the control, DM, *C. afer*, DM +MTF and DM +*C. afer* groups were 0.48 ± 0.00 , 0.48 ± 0.06 , 0.14 ± 0.00 , 0.80 ± 0.00 and 1.18 ± 0.04 (Table 6 and figure 9). There was a significant reduction and increase in the *C. afer* and DM+MTF and DM+ *C. afer* group when compared to the control and DM groups. Again, DM+MTF and DM+*C. afer* groups were significantly higher than *C. afer* groups only, while the DM+*C. afer* group had a significant increase compared to DM+MTF group only.

Table 6: Comparison of aqueous extract of *C. afer* leaves on total, conjugated, unconjugated bilirubin and cholesterol levels in control and experimental groups.

Groups	TB	CB	UB	Level of Significance P value (p<0.05)
Control	1.74 ± 0.06	1.26 ± 0.13	0.48 ± 0.00	-
DM	2.12 ± 0.06	1.64 ± 0.12	0.48 ± 0.06	No significance
<i>C. afer</i>	$1.46 \pm 0.10a$	1.32 ± 0.11	$0.14 \pm 0.00^*a$	*a vs control and DM
DM+MTF	$2.48 \pm 0.13^*b$	1.68 ± 0.12	$0.80 \pm 0.00b$	*b vs control and <i>C. afer</i> .
DM+ <i>C. afer</i>	$2.42 \pm 0.13^*b$	1.24 ± 0.09	$1.18 \pm 0.04^*abc$	*abc vs control, DM, <i>C. afer</i> and DM+MTF

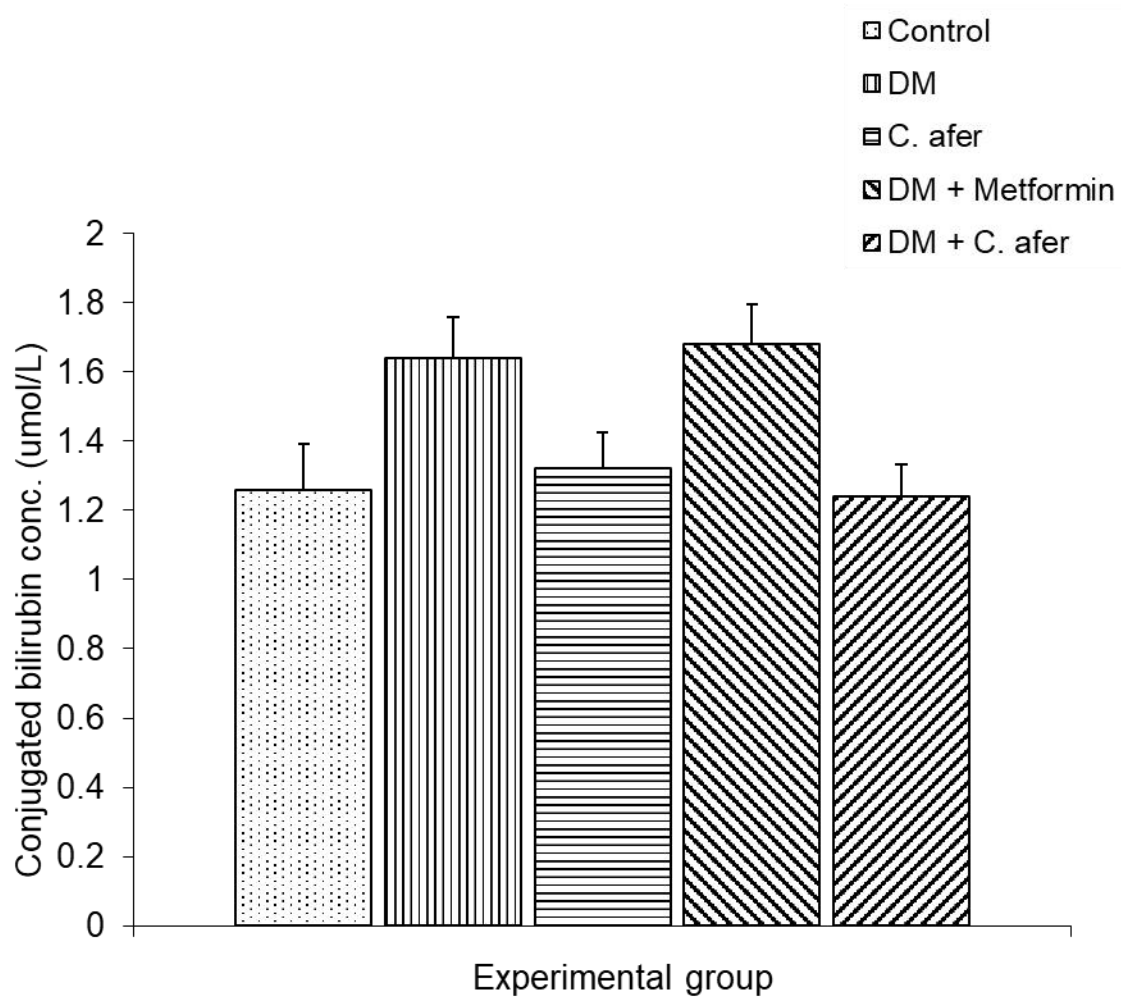


FIG. 8: Conjugated bilirubin concentration in the different experimental groups.

Values are expressed as mean +SEM, n = 7.
No significant differences among groups

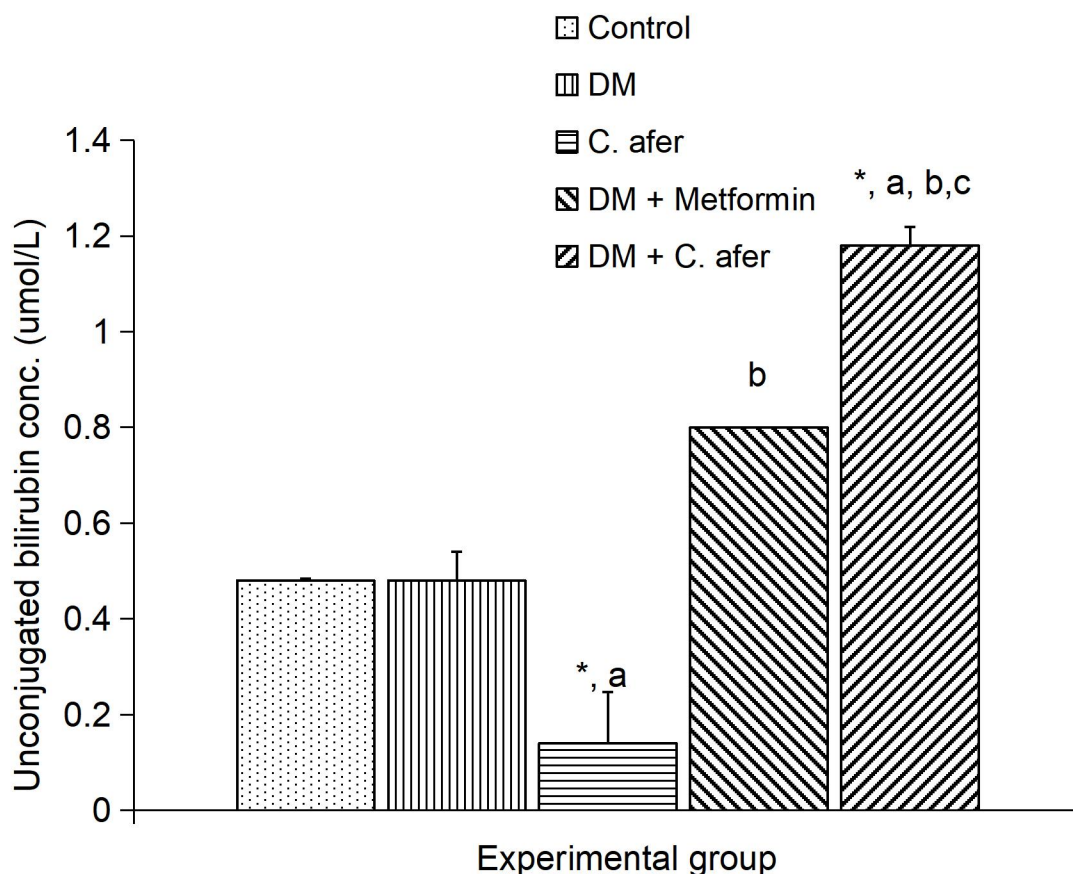


FIG. 9: Unconjugated bilirubin concentration in the different experimental groups.

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*

DISCUSSION

Streptozotocin is a chemical agent that damages the DNA of the pancreatic islets through a free radical mechanism. According to Lenzen [21], destruction of the pancreatic cells will impair glucose utilization and increase free radical production, thus the reason for the initial high glucose concentration in the DM groups after treatment with STZ. After a period of treatment of 28 days the final glucose

concentration was reduced in DM + MTF groups and DM + *C. afer* groups compared to the DM untreated group. This probably is attributed to the extract ability to restore the destroyed pancreatic cells and enhance glucose utilization. Several studies attest 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. 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* [22,23,24]. Hence, the probable reason for the reduced blood glucose concentration seen after treatments in the *C. afer* group. Alterations in bile secretion and composition have been attributed to different biliary diseases such as cholelithiasis and abnormal fat metabolism [25]. Evaluation of biliary flow rate showed a non-significant difference in experimental groups compared to the control groups, while there was a distortion of biliary electrolytes. Na^+ , Cl^- and K^+ was significantly increased in the *C. afer* group and DM + *C. afer* group compared to the control, while HCO_3^- was significantly reduced in the DM and *C. afer* groups only. Normally reabsorption of electrolytes occurs in the gall bladder (except potassium and calcium) and cholesterol occurs in the gallbladder, thus making the substances more concentrated [26]. Bicarbonate (HCO_3^-) secretion is primary function of cholangiocytes, which functions through cAMP or cytosolic Ca^{2+} ions [27]. These cholangiocytes are heterogeneous, highly dynamic populated epithelial cells that line the epithelial of the biliary tree to aid HCO_3^- secretion. The reduced HCO_3^- concentration observed in the DM group and *C. afer* group could probably have been as a result of destructions of the cholangiocyte cells. Since DM is known to produce free radicals which probably resulted in these oxidative stress damages. This finding supports the study by Ani *et al.* [28], where bicarbonate ions were reduced due to similar phytochemical in plant such as alkaloids which are also found in *C. afer*.

The potassium ion concentration was significantly higher in the *C. afer* group, DM

+ *C. afer* and DM + MTF group while the chloride ion concentration followed similar pattern except for the DM + *C. afer* which was not significantly increased when compared to the control. However, there was a significant increase in Na^+ ion concentration in the DM + MTF group as opposed to the reduction seen in the Diabetic untreated, DM + *C. afer* group. The second secretory phase in the production of bile involves the storage in the gall bladder where electrolyte such as Na^+ , Cl^- and water are constantly absorbed through the gall bladder mucosa, concentrating the bile [29]. Most likely, the distortion seen in experimental group treated with *C. afer* could be as a result of phytochemical such as phenols, saponins, tannins and alkaloids which have been shown to have modulatory effect on biliary flow rate and electrolyte constituent by stimulating effect of Secretin [30].

Spontaneous contraction of ileum of the rats in the control and experimental groups were measured, the DM untreated and DM+*C. afer* group showed a significant increase in both motility and transit time compared to the control groups, while the DM+MTF group had no significant difference, this signifies *C. afer* increases GI motility and transit compared to the reference drug MTF in DM+MTG group which had similar GI motility and transit as compared to the corresponding control. Nonetheless, a study by Ijeoma *et al.* [31] suggests *C. afer* leaves extracts contain phytochemicals of both anticholinergic and antihistamine properties and as such capable of exerting antispasmodic effects on the gastrointestinal tract, Momoh *et al.* [32] had reported that *C. afer* contain phytochemical substances including alkaloids, flavonoids, tannins, phenols, glycosides and terpenoids, some of which have been implicated in the inhibition of intestinal motility and control of diarrhea by increasing colonic water and electrolyte reabsorption.

Small contractions of acetylcholine (Ach) produced a gradual dose dependent increase in the heights of the contractions of the control and experimental groups. The control group had the highest response to ach followed by the DM + *C. afer* group untreated group, and then *C. afer* group. While administration of atropine showed a significant reduction in all groups with the *C. afer* group had the highest reduction in contraction, this suggest the contractility by *C. afer* could be through the Muscarinic receptors. Furthermore, administration of propranolol caused a significant reduction and increase in contractility in both *C. afer* and DM+ *C. afer* groups respectively. The reason for this variance in response is unclear.

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, diabetic suffer from frequent taste 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 [33], hence the composition of these electrolytes might have even contributed to increased water consumption, hence the significance increased 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*, again 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 [23].

Fecal weight was reduced in *C. afer*, DM +MTF and DM+ *C. afer* groups compared to the DM untreated groups. This again signifies a modulation in fecal weight by *C. afer* similar to the reference drug metformin. From our initial result, we can presume, fecal weight is proportional to feed intake, hence the high fecal weight seen in the DM group, while *C. afer* and MTF was able to reduce this feed intake by causing glucose utilization and abating the polyhagic effect of DM, thus, the reduced fecal weight seen in these groups. Conversely, in DM group water intake was inversely related to fecal moisture, this only depicts high water absorption due to polyphagia, and this correlates with gut fluid transfer which was higher in the DM untreated group, whilst other experimental groups were significantly lower compared to the DM group. Likewise, fecal moisture was higher in *C. afer* and DM+ *C. afer* groups compared to DM groups. This signified low absorption of fluid or possibly high fluid intake caused by *C. afer*.

Fecal glucose concentration was significantly reduced and increased respectively in *C. afer* and DM +MTF groups respectively, compared to the control, while DM+ *C. afer* group was significantly increased compared to *C. afer* group. The inverse relationship between *C. afer* and DM+ *C. afer* group can again to be attributed to the glucose content which accompanied polyphagia in the later. Depending on the food composition, the site of the gastrointestinal tract and time of the day, the postprandial glucose concentrations in the GIT lumen can vary in a large range and can be several times higher than in the blood. In rats fed with 65% glucose diet, the highest concentration of free glucose was found in the stomach [34]. This shows that the gut provides both a barrier by limiting glucose

passage and a regulatory mechanism for maintaining blood glucose levels. Thus, glucose absorption together with glucose ingestion and metabolism are all the interconnected processes determining the blood glucose levels and its availability to organs and tissues [35]. Evaluation of fecal electrolytes showed varied results in all groups, K^+ , HCO_3^- was significantly reduced in DM+C. *afer* group while Cl^- was significantly increased in DM+ C. *afer* group compared with the corresponding control, which signifies poor absorption or high cl^- content of C. *afer*. While Na^+ was increased only in C. *afer* group. This array of distortion of electrolytes predisposes to cholelithiasis, since electrolyte imbalance can lead to gallstone formation [36]. Ergo, these electrolytes again might have originated from the bile and appeared in feces.

CONCLUSION

In conclusion, C. *afer* demonstrated promising antidiabetic and gastrointestinal modulating effects in diabetic rats. The extract significantly reduced blood glucose levels and improved glucose absorption, likely due to its bioactive compounds like diosgenin and aferosides. Altered biliary electrolyte concentrations and increased gut motility were observed, indicating potential effects on bile secretion and gastrointestinal function. Additionally, fecal weight and glucose concentrations were modified, with C. *afer* influencing fluid and electrolyte balance, potentially contributing to reduced polyphagia and improved glucose utilization. Despite these benefits, electrolyte imbalances suggest possible risks like cholelithiasis.

REFERENCES

1. Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., et al.; IDF Diabetes Atlas Committee. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Research and Clinical Practice*, 157, 107843.
2. Jones, K. L., Russo, A., Berry, M. K., Stevens, J. E., Wishart, J. M., & Horowitz, M. (2002). A longitudinal study of gastric emptying and upper gastrointestinal symptoms in patients with diabetes mellitus. *American Journal of Medicine*, 113(6), 449–455.
3. Koch, C. A., & Uwaifo, G. I. (2008). Are gastrointestinal symptoms related to diabetes mellitus and glycemic control? *European Journal of Gastroenterology & Hepatology*, 20(9), 822–825.
4. Sumida, Y., & Yoneda, M. (2018). Current and future pharmacological therapies for NAFLD/NASH. *Journal of Gastroenterology*, 53(2), 362–376.
5. Yki-Järvinen, H. (2014). Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. *The Lancet Diabetes & Endocrinology*, 2(11), 901–910.
6. Inzucchi, S. E., Bergenstal, R. M., Buse, J. B., et al. (2015). Management of hyperglycemia in type 2 diabetes, 2015: A patient-centered approach. *Diabetes Care*, 38(1), 140–149.
7. Goodies, M. E., Emmanuel, I. E., Matthew, O. J., Tedwins, E. J. O., Lotanna, A. D., Earnest, E. O., et al. (2015). Antidiabetic activity and toxicity evaluation of aqueous extracts of *Spondias mombin* and *Costus afer* on Wistar rats. *Journal of Pharmaceutical Research International*, 6(5), 333–342.
8. Okwuanga, N. L., Onyewuchi, M. O., Mbah, C. A., Okafor, E. C., Ejikeme, S. N., Nwanama, E. K., et al. (2020). Hepato-protective effect of *Costus afer* stem juice extract on alcohol-induced liver

- toxicity in adult female Wistar rats. *Journal of Complementary and Alternative Medical Research*, 25(5), 10–17.
9. Okafor-Onyia, S. D., Nwinyi, O. C., Vandi, A., et al. (2015). Proximate composition, mineral content and in-vitro antioxidant activity of leaf and stem of *Costus afer*. *Journal of Intercultural Ethnopharmacology*, 3(3), 128–134.
 10. Boison, D., Adinortey, C. A., Babanyinah, G. K., Quasie, O., Agbeko, R., Wiabo-Asabil, G. K., et al. (2019). *Costus afer*: A systematic review of evidence-based data in support of its medicinal relevance. *Scientifica*, 2019, 3732687.
 11. Ezejiofor, A. N., Igweze, Z. N., Udowelle, N. A., & Orisakwe, O. E. (2017). Histopathological and biochemical assessments of *Costus afer* stem on alloxan-induced diabetic rats. *Journal of Basic and Clinical Physiology and Pharmacology*, 28(4), 383–391.
 12. Vickers, S., Duncan, C. A., Slaughter, D. E., Arison, B. H., Greber, T., Olah, T. V., & Vyas, K. P. (1998). Metabolism of MK-499, a class III antiarrhythmic agent, in rats and dogs. *Drug Metabolism and Disposition*, 26(5), 388–395.
 13. Forrester, R. L., Wataji, L. J., Silverman, D. A., & Pierre, K. J. (1976). Enzymatic method for determination of CO₂ in serum. *Clinical Chemistry*, 22(2), 243–245.
 14. Kolkolf, I. M., & Elving, J. (1967). *Treatise on analytical chemistry* (Vol. 1, pp. 1–20). New York.
 15. Sherlock, S. (1951). Determination of bilirubin levels. In *Liver Disease* (p. 204). Churchill, London.
 16. Allain, C. C., Poon, L. S., Chan, C. S., Richmond, W. F. P. C., & Fu, P. C. (1974). Enzymatic determination of total serum cholesterol. *Clinical Chemistry*, 20(4), 470–475.
 17. Makinde, M. O., & Bobade, P. A. (1994). Osmotic fragility of erythrocytes in clinically normal dogs and dogs infected with parasites. *Research in Veterinary Science*, 57(3), 343–348.
 18. Folch, J., Lees, M., & Stanley, G. S. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry*, 226(1), 497–509.
 19. Kamei, M., Ohgaki, S., Kanbe, T., Niiya, I., Mizutani, H., Matsui-Yuasa, I., ... & Morita, S. (1995). Effects of highly hydrogenated soybean oil and cholesterol on plasma, liver cholesterol, and fecal steroids in rats. *Lipids*, 30(6), 533–539.
 20. Esmerino, L. A., Ranali, J., & Rodrigues Jr, A. L. (1998). Blood glucose determination in normal and alloxan-diabetic rats after administration of local anesthetics containing vasoconstrictors. *Brazilian Dental Journal*, 9(1), 33–37.
 21. Lenzen, S. (2008). The mechanisms of alloxan-and streptozotocin-induced diabetes. *Diabetologia*, 51(2), 216–226.
 22. Uwah, A. F., Ewere, E. G., & Ndem, J. I. (2015). Hypoglycemic and haematologic effects of crude stem juice of *C. afer* on alloxan-induced diabetic Wistar rats. *American Journal of Ethnomedicine*, 2(4), 2348–9502.
 23. Jesus, M., Martins, A. P., Gallardo, E., & Silvestre, S. (2016). Diosgenin: Recent highlights on pharmacology and analytical methodology. *Journal of Analytical Methods in Chemistry*, 2016, 4156293. <https://doi.org/10.1155/2016/4156293>
 24. Lin, R. C., Lacaille-Dubois, M. A., Hanquet, B., Correia, M., & Chauffert, B. (1997). New diosgenin glycosides from *C.*

- afer*. *Journal of Natural Products*, 60(11), 1165–1169.
25. Ofem, O. E., Ikpi, D. E., & Antai, A. B. (2014). Altered biliary flow rate and bile composition following consumption of ethanolic fruit extract of *Dennettia* in rats. *International Journal of Applied and Basic Medical Research*, 4(1), 20.
26. Ofem, O. E., Ikpi, D. E., & Essien, N. M. (2013). Increased bile flow rate and altered composition of bile induced by ethanolic leaf extract of *Azadirachta indica* (neem) in rats. *Nigerian Journal of Experimental and Clinical Biosciences*, 1(1&2), 18–22.
27. Jones, H., Alpini, G., & Francis, H. (2015). Bile acid signaling and biliary functions. *Acta Pharmaceutica Sinica B*, 5(2), 123–128.
28. Ani, O. N., Udedi, S. C., Akpata, E. I., Ezeigwe, O. C., Oguazu, C. E., Onyishi, C. K., & Nwakaudu, E. N. (2020). Effects of ethanol leaf extract of *Justicia carnea* on biochemical indices of alloxan-induced diabetic rats. *IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)*, 6(2), 39–46.
29. Hall, J. E., & Hall, M. E. (2020). *Guyton and Hall Textbook of Medical Physiology* (E-book). Elsevier Health Sciences.
30. Feng, F., Liu, X., Sun, C., Yang, X., Fang, Y., & Li, S. (2020). Exogenous secretin improves parenteral nutrition-associated liver disease in rats. *Journal of Pediatric Gastroenterology and Nutrition*, 70(4), 430–435.
31. Ijeoma, S. N., Nwankwo, A. A., Emelike, C. U., & Nwankudu, O. N. (2014). Antispasmodic activity of *C. afer* leaves extract on an isolated rabbit jejunum. *Central European Journal of Experimental Biology*, 3(3), 22–26.
32. Momoh, S., Yusuf, O. W., Adamu, M. M., Agwu, C., & Atanu, F. O. (2011). Evaluation of the phytochemical composition and hypoglycaemic activity of methanolic leaves extract of *Costus afer* in albino rats. *British Journal of Pharmaceutical Research*, 1(1), 1–8.
33. Boison, D., Adinortey, C. A., Babanyinah, G. K., Quasie, O., Agbeko, R., Wiabo-Asabil, G. K., & Adinortey, M. B. (2019). *C. afer*: A systematic review of evidence-based data in support of its medicinal relevance. *Scientifica*, 2019, 3732687. <https://doi.org/10.1155/2019/3732687>
34. Ferraris, R. P., Yasharpour, S. A., Lloyd, K. C., Mirzayan, R. A., & Diamond, J. M. (1990). Luminal glucose concentrations in the gut under normal conditions. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 259(5), G822–G837.
35. Gromova, L. V., Fetissov, S. O., & Gruzdkov, A. A. (2021). Mechanisms of glucose absorption in the small intestine in health and metabolic diseases and their role in appetite regulation. *Nutrients*, 13(7), 2474. Retrieved from <http://dx.doi.org/10.3390/nu13072474>
36. Lambou-Gianoukos, S., & Heller, S. J. (2008). Lithogenesis and bile metabolism. *Surgical Clinics of North America*, 88(6), 1175–1194.