Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.10, No.6, 2020

Hepatic- and Renal protective Effects of Chrysophyllum albidum (African Star Apple) Fruit-Skin Supplementation on Streptozotocin-Induced Diabetic Rats

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Abstract
Chrysophyllum albidum commonly called African star apple, has been reported for its antidiabetic potential but its effect on liver and kidney functions in diabetic rats has not been well investigated. This study investigated effects of *Chrysophyllum albidum* fruit-skin (CAFS) supplemented diet on Streptozotocin-induced diabetic rats. Diabetes was induced intraperitonealy at a single dose of 50mg/kg bw of Streptozotocin (STZ). Forty male Wistar rats weighing averagely 170g were randomly divided into four groups of ten rats each; Group 1-Normal control, Group 2-Diabetic control, Group 3-Diabetic rats treated Glibenclamide, Group 4-Diabetic rats treated 70g/kg CAFS supplemented diet. After four weeks of treatment, the rats were sacrificed. Liver function biomarkers (Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP)) activities, kidney function biomarkers (Creatinine and Urea), electrolyte ions (K^+, Na^+, HCO_3^- and Cl^-), total protein and bilirubin levels were measured in the plasma. The diabetic control exhibited hepato-renal dysfunction with significant ($p<0.05$) increase in the liver marker enzymes' activities, total bilirubin, creatinine and urea concentrations while decreasing total protein, albumin and electrolyte ions levels compared with normal control group. Treatment with CAFS supplemented diet significantly ($p<0.05$) enhanced liver and kidney recovery by reducing the elevated activities of liver marker enzymes, reversed the increments in creatinine, urea and total bilirubin while increasing the concentration of total protein and restoring electrolyte ions concentrations to near normal values. CAFS supplemented diet has hepatic- and renal protective effects on STZ-induced diabetic rats. Thus, CAFS could be considered as therapeutic agent against the progression of diabetes complications.
Keywords: *Chrysophyllum albidum*; fruit-skin; liver markers; kidney markers; diabetes.
DOI: 10.7176/JNSR/10-6-02
Publication date: March 31st 2020

1.0 Introduction

Diabetes mellitus (DM) is a major global public health problem with an escalating incidence and prevalence, particularly in developing and newly industrialized countries (Danaei *et al.*, 2011). Globally, in the year 2015, 415 million people were estimated to have diabetes and by 2040, the prevalence is projected to increase to 642 million (International Diabetes Federation, 2015). One of the most important clinical features of diabetes is its associated complications, which can affect multiple vital organ systems, thereby leading to more severe and irreversible pathological conditions such as nephropathy, retinopathy, hepatopathy, vasculopathy, neuropathy and cardiovascular diseases (Reid, 2006). The duration and severity of hyperglycemia is the major causative factor in initiating organ damage.

Researches indicate that, DM is associated with a number of liver abnormalities that affect glycogen and lipid metabolism (Sanchez *et al.,* 2000; Bolkent *et al.,* 2004; Koyuturk *et al.,* 2005). Our previous study demonstrated that persistent hyperglycemia caused significant reduction in the liver glycogen levels and elevation of plasma and hepatic lipids (except high-density lipoprotein (HDL)) contents in diabetic rats (Ibrahim *et al.,* 2019b). Another studies by Chiang *et al.* (2011), Birkenfeld and Shulman (2014) as well as Nakahara *et al.* (2014) found that DM causes liver disease, such as hepatic steatosis, nonalcoholic steatohepatitis, fibrosis and cirrhosis. A fatty liver and hyperglycaemia candestroy the hepatocytes and contribute to increased morbidity and mortality among diabetic patients (Levinthal and Tavill, 1999).

Kidney disease attributed to diabetes is a major but under-recognized contributor to the global burden of disease. As reported by Reutens (2013), United States Renal Data System Annual Data Report (2015) and Papadopoulou-Marketou *et al.* (2018), diabetic kidney disease (DKD) develops in approximately 30% of patients with type1 diabetes mellitus (DM1) and approximately 40% of patients with type 2 diabetes mellitus (DM2). Its increasing prevalence parallels the dramatic worldwide rise in prevalence of diabetes (De Boer *et al.,* 2011, World

Health Organization, 2014) and is considered the single strongest predictor of mortality in patients with diabetes (Maisonneuve *et al.,* 2000, United States Renal Data System, 2003). Development of DKD is associated with many alterations in the structure of multiple kidney compartments, which lead to glomerular hypertrophy, glomerulo sclerosis, tubulointerstitial inflammation and fibrosis. The most characteristic marker of DKD is albuminuria, which is associated with renal disease progression and cardiovascular events (Gross *et al.,* 2005). The Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation trial showed that intensive glucose control could reduce development of microalbuminuria and macroalbuminuria *(Perkovic et al., 2013).*

In recent times, attentions are being drawn to complementary and alternative treatments for diabetes focusing on functional foods and their bioactive compounds (Tahrani *et al.,* 2010; Karigidi and Olaiya, 2019). *Chrysophyllum albidum*, commonly called African star apple is one of such functional foods, which belongs to Sapotaceae family and locally called 'agbalumo' in Yoruba,'agwaluma' in Hausa and 'udara' in Ibo tribes of Nigeria (Amusa *et al.,*2003; Bello and Henry, 2015). It is distributed in the tropical rain forest and coastal region of West Africa (Amusa *et al.,* 2003). *C. albidum* fruit (Figure 1) is seasonal, found between December and April. Its fruit skin or peel is orange to golden yellow in colour when ripe and is chewy like gum with sweet/sour taste. The fruit-pulp has application in jams and jellies and can compete with raspberry jams and jellies (Okafor, 1975, Umelo, 1997).

Figure 1: *Chrysophyllum albidum* fruits

Source: Orwa *et al.* (2009)

Studies on different parts of *C. albidum* plant showed the antioxidants nature of the plant (Idowu *et al.,* 2006; Okoli and Okere, 2010; Adebayo *et al.,* 2011b; Imaga and Urua, 2013; Omotosho *et al.,* 2013; Egharevba *et al.,* 2015), which can support health by scavenging free radicals and prevent oxidative stress related diseases (Burits and Bucar, 2002).Olorunnisola *et al.* (2008) reported the hypolipidemic and hypoglycemic properties of *C. albidum* seed cotyledon and Adebayo *et al.* (2011a) demonstrated the hepatoprotective effect of *C. albidum* leaf extract against carbon tetrachloride (CCl_4) induced liver damage in Wistar rats. Previously, we reported the antioxidant effect of *C. albidum* fruit skin extracts that was associated with flavoniods and phenolic contents (Ibrahim *et al.,* 2019a). In addition, the hypolipidemic and antihyperglycemic potentials of *C. albidum* fruit skin in STZ-induced diabetic rats was demonstrated (Ibrahim *et al.,* 2019b). The aim of the present study was to explore the effect of *C. albidum* fruit skin supplementation on liver and kidney function parameters of STZ-induced diabetic rats.

2.0Materials and methods

2.1 Chemicals

All chemicals and drug used were of analytical grade. Chemicals, drug and solvents were purchased from Sigma Chemical Co. (St Louis, MO, USA). Assay kits purchased from Randox Laboratories Limited, UK were used for the analysis of liver and renal function parameters.

2.2 Collection and preparation of *C. albidum* fruit skin

The *C. albidum* fruits were purchased in Moniya market, Akinyele local government area of Oyo State, South-Western Nigeria and washed. The fruit skin was separated, cut into small pieces and lyophilized using Lyophilizer

Millorock Bench-Top Freeze Dryer, Germany. Lyophilized sample was stored at -20ºC until further use.

2.3 Feed formulation

Two diets were formulated according to the recommendations of National Academy of Sciences (1995) on nutrients requirement for laboratory rats for growth and maintenance as described in our previous study (Ibrahim *et al*., 2019b). Normal control (NC) diet, which is free of *C. albidum* fruit-skin (CAFS) and Test (CAFS) diet, which was supplemented with 70g/kg CAFS. The two feed formulations were made into pellets to appeal to the rats.

2.4 Experimental animals

Male Wistar rats weighing averagely 170g were obtained from the animal house of the Department of Physiology, Ben Carson (Snr) School of Medicine, Babcock University, Ilishan-Remo, Nigeria. Rats were fed with commercial pellets and water *ad libitum* during the two weeks of acclimatization under standard laboratory conditions. After induction of DM, they were treated with CAFS supplemented diet for four weeks. All experiments were conducted in compliance with ethical guide for care and use of laboratory animals of the Babcock University Health Research Ethics Committee (BUHREC).

2.5 Induction of Diabetes Mellitus

Diabetes was induced in overnight fasted rats by a single intraperitoneal injection of STZ (50 mg/kg bodyweight (bw)) dissolved in freshly prepared citrate buffer (0.1 M, pH 4.5). After 72 h of STZ administration, fasting blood glucose (FBG) levels were checked using an Accu-Chek glucometer and rats with FBG≥ 250 mg/dL were considered diabetic and used in the present study.

2.6 Experimental design

Forty (40) male Wistar rats were randomly divided into 4 groups of 10 rats each.
Group 1: Non-diabetic rats, served as normal control group.
Group 2: Diabetic untreated rats, served as diabetic control group.
Group 3: Diabetic rats, received 2.5mg/kg bw glibenclamide as a standard drug in 1 ml normal saline orally by intragastric tube once in a day for four weeks, served as glibenclamide treated diabetic rats
Group 4: Diabetic rats, fed with a 70g/kg freeze dried-*Chrysopyllum albidum* fruit skin (CAFS) supplemented diet, served as CAFS treated diabetic rats.
In addition, rats in groups 1-3 were fed with the formulated NC diet (free of CAFS) and groups 1, 2 & 4 received only 1 ml normal saline orally by intragastric tube once in a day for four weeks while given water *ad libitum* to all the groups.

2.7 Blood collection and preparation of sample

After the four weeks of experimental period, rats were sacrificed and blood sample was collected by cardiac puncture into lithium-heparinized bottles. It was centrifuged at 10,000 x g for 15 minutes to obtain the plasma, which was used for the analysis of liver and renal function parameters.

2.8 Determination of liver and renal function parameters

The liver function parameters and renal markers (Urea and creatinine) concentrations in the plasma were estimated using Randox assay Kits (Randox Laboratories Ltd, UK).The electrolyte ions (Na^+, K^+, Cl^- and HCO_3^-) concentrations were estimated using ion selective electrode, ISE analyzer (Audicom AC9900 automatic electrolyte analyzer). The methods of Reitman and Frankel (1957) were employed to estimate aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in the plasma. The procedure described by Kind and King (1954) was used to estimate alkaline phosphatase activities while the methods of Tietz (2012) were used to determine the total protein and direct bilirubin (conjugated bilirubin) contents of the sample. Total bilirubin was determined colorimetrically according to the method described by Jendrassik and Grof, (1938) while albumin was determined using the method described by Weichselbaum, (1946) and Henry, (1974). The procedure of Fawcett and Scott (1960) was used to determine urea concentration while creatinine concentration was determined by the method of Boneses and Taussk (1945).

Percentage (%) decrease was calculated using the formula:

$$\frac{\text{Diabetic Control (DC)} - \text{Treatment (T)}}{\text{Diabetic Control (DC)}} \times 100 \quad (1)$$

Percentage (%) increase was calculated using the formula:

$$\frac{\text{Treatment (T)} - \text{Diabetic Control (DC)}}{\text{Diabetic Control (DC)}} \times 100 \quad (2)$$

2.9 Statistical analysis

The obtained data was analyzed using standard Statistical Software Package for Social Sciences (SPSS) for Window XP Software Programme (version 13.0). Group comparisons were done using one way analysis of variance (ANOVA) test. Significant differences between control groups and experimental groups were assessed by Least Significant Difference (LSD) to test the significance at $p< 0.05$. All data were expressed as Mean ± Standard error of the mean (SEM), n=6.

3.0 Results

3.1 Effect of CAFS on liver function parameters

The protective effects of CAFS supplementation on liver function parameters in STZ-induced diabetic rats were shown in (Figures 2- 9). It was observed that induction of diabetes significantly ($p < 0.05$) increased the activities of plasma AST (Figure 2) from 19.87±1.05 to 59.22±2.17U/L, ALT (Figure 3) from 18.70±0.97 to 28.49±2.31U/L, ALP (Figure 4) from 288.51±1.98 to 911.16±2.27 U/L and AST:ALT (Figure 5) from 1.06±0.003 to1.80±0.14 compared to rats in the control group. However, treatment of diabetic rats with both CAFS supplemented diet and glibenclamide (standard drug) resulted in the suppression of elevated activities of plasma AST by 52% in both CAFS and glibenclamide-treated rats and ALP by 59% and 52% in CAFS and glibenclamide-treated rats respectively while the elevated activity of ALT in the two treatment groups were mildly suppressed. Feeding of a 70g/kg CAFS supplemented diet to diabetic rats for four weeks exhibited similar ameliorative effects with daily administration of 2.5mg/kg bw glibenclamide to diabetic rats for four week of treatment.

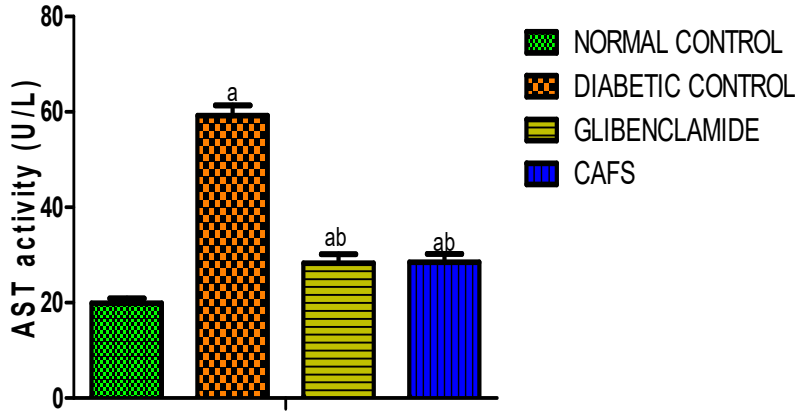

Figure 2: Effect of CAF supplemented diet on AST activity of streptozotocin-induced diabetic rats.
'a' is significantly different from normal control at ($p < 0.05$), **'b'** is significantly different from diabetic control at ($p < 0.05$).

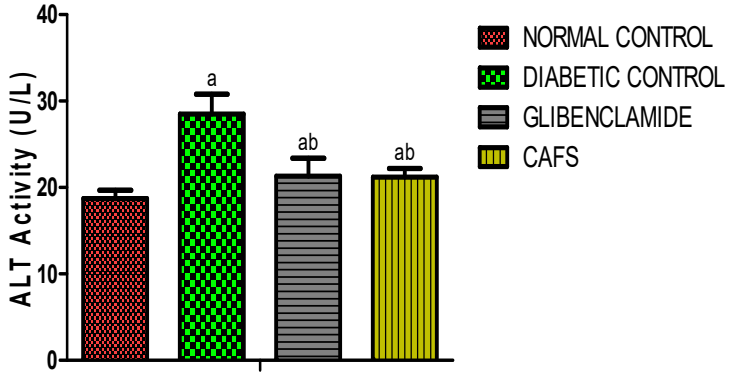

Figure 3: Effect of CAF supplemented diet on ALT activity of streptozotocin-induced diabetic rats.
'a' is significantly different from normal control at ($p < 0.05$), **'b'** is significantly different from diabetic control at ($p < 0.05$).

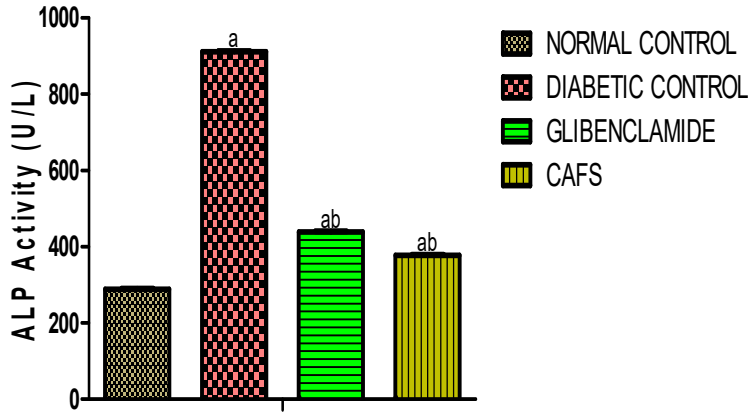

Figure 4: Effect of CAF supplemented diet on Alkaline phosphatase (ALP) of streptozotocin-induced diabetic rats.
'a' is significantly different from normal control at ($p < 0.05$), **'b'** is significantly different from diabetic control at ($p < 0.05$).

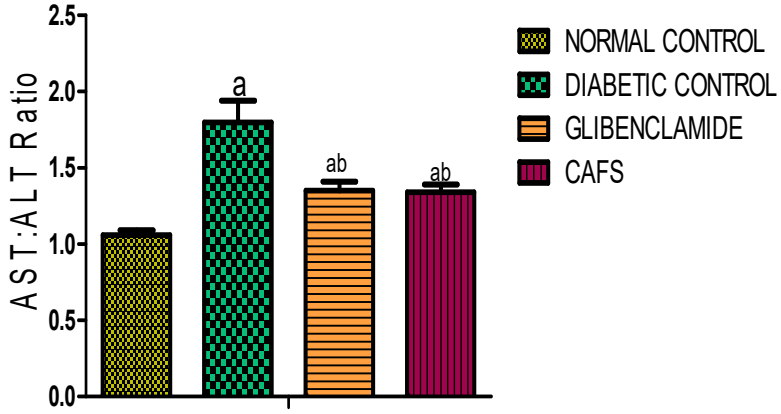

Figure 5: Effect of CAF supplemented diet on AST: ALT ratio of streptozotocin-induced diabetic rats.
'a' is significantly different from normal control at ($p< 0.05$), **'b'** is significantly different from diabetic control at ($p < 0.05$).

Similar to the trend of liver marker enzymes, the total bilirubin (Figure 6) and unconjugated bilirubin (Figure 7) levels were also significantly ($p < 0.05$) increased in diabetic untreated rats from 0.73±0.07 to 1.15±0.10mg/dl and 0.23± 0.04 to 0.82 ± 0.09mg/dl respectively compared to rats in the control group. However, the increment observed in both total - and unconjugated bilirubins were reversed by 32% & 63% in diabetic rats treated with CAFS supplemented diet and by 29% & 55% in diabetic rats treated with glibenclamide. In addition, the present study also showed that diabetes state significantly ($p < 0.05$) decrease the plasma levels of conjugated bilirubin from 0.50±0.04 mg/dl to 0.33±0.02 mg/dl (Figure 6) and total protein from 8.95±0.09 g/dl to 5.16±0.03g/dl (Figure 8). Treatment of diabetic rats with both CAFS supplemented diet and glibenclamide; however inhibited these suppressions by 45% and 36% respectively for conjugated bilirubin and 55% in the two treatments.

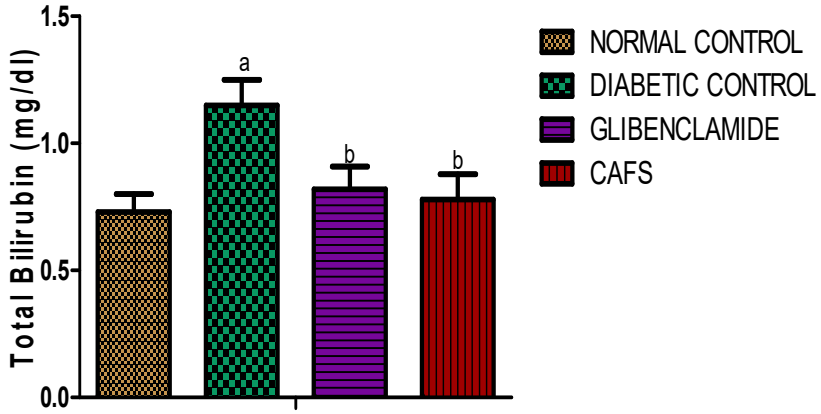

Figure 6: Effect of CAF supplemented diet on Total bilirubin of streptozotocin-induced diabetic rats.
'a' is significantly different from normal control at ($p < 0.05$), '**b**' is significantly different from diabetic control at ($p < 0.05$).

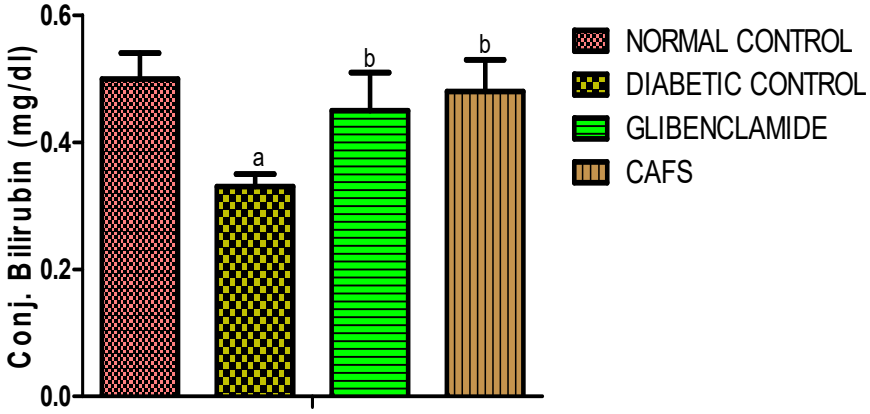

Figure 7: Effect of CAF supplemented diet on conjugated bilirubin of streptozotocin-induced diabetic rats.
'a' is significantly different from normal control at ($p < 0.05$), **'b'** is significantly different from diabetic control at ($p < 0.05$).

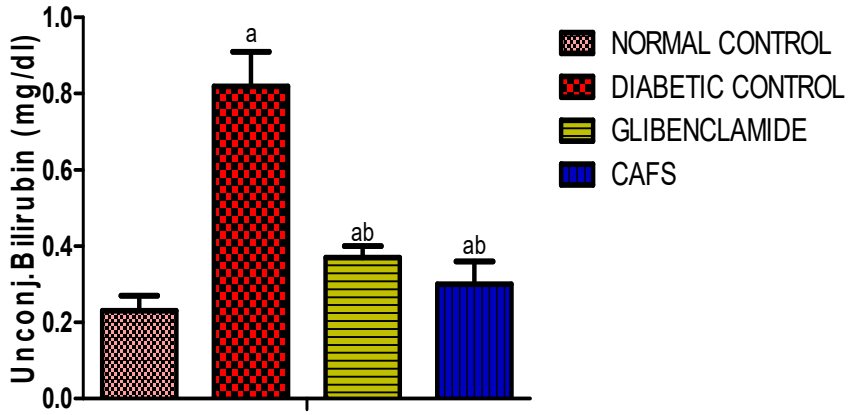

Figure 8: Effect of CAF supplemented diet on unconjugated bilirubin of streptozotocin-induced diabetic rats.
'a' is significantly different from normal control at ($p < 0.05$), **'b'** is significantly different from diabetic control at ($p < 0.05$).

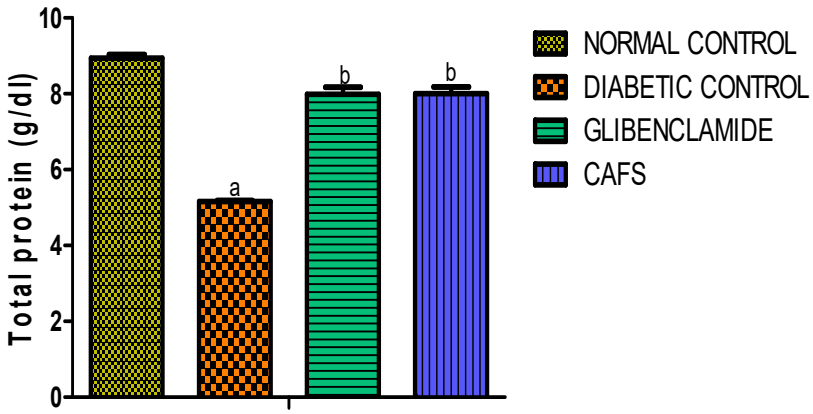

Figure 9: Effect of CAF supplemented diet on Total protein of streptozotocin-induced diabetic rats.
'a' is significantly different from normal control at ($p < 0.05$), **'b'** is significantly different from diabetic control at ($p < 0.05$).

3.2 Effect of CAFS on Renal function parameters

The changes in plasma concentration of Creatinine, Urea, Albumin, Globulin and Albumin: Globulin ratio of STZ-induced diabetic rats fed with and without CAFS supplemented diet are presented in Figures 10-14. It was observed that induction of diabetes was associated with significant ($p<0.05$) increase in the plasma levels of creatinine(1.06±0.04mg/dl to 1.18 ± 0.01mg/dl; Figure 10) and urea (42.07±1.98mg/dl to 85.23 ± 3.03mg/dl; Figure 11). On the contrary, diabetes state resulted in decreased plasma levels of albumin (5.23±0.32g/dl to 1.91±0.16g/dl; Figure 12), Globulin (3.71 ± 0.30g/dl to 3.24 ± 0.17g/dl; Figure 13) and Albumin: Globulin ratio (1.48 ± 0.20 to 0.60± 0.07; Figure 14) relative to rats in the control group. Treatment of diabetic rats with both CAFS supplemented diet and glibenclamide for four weeks however, reversed the elevations in creatinine by 8% and 10% respectively and in urea by 35% in the two treatments while albumin, globulin and Albumin: Globulin ratio were restored significantly ($p < 0.05$) to near normal values.

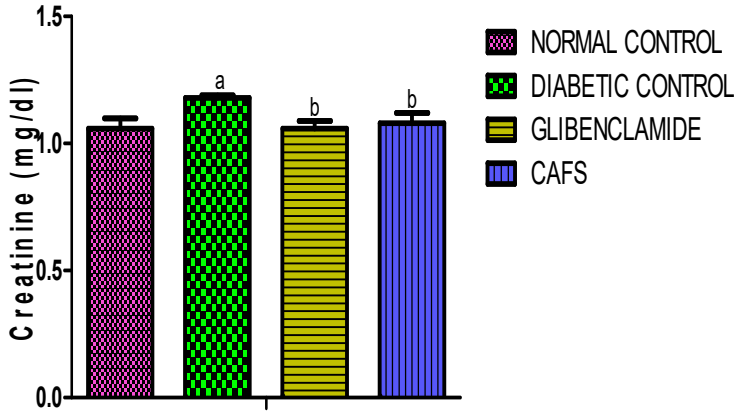

Figure 10: Effect of CAF supplemented diet on Creatinine concentration of streptozotocin-induced diabetic rats.
'a' is significantly different from normal control at ($p < 0.05$), **'b'** is significantly different from diabetic control at ($p < 0.05$).

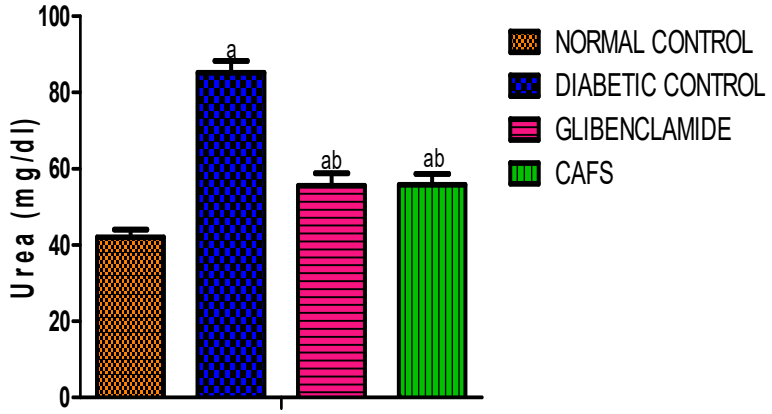

Figure 11: Effect of CAF supplemented diet on Urea concentration of streptozotocin-induced diabetic rats. **'a'** is significantly different from normal control at ($p < 0.05$), **'b'** is significantly different from diabetic control at ($p < 0.05$).

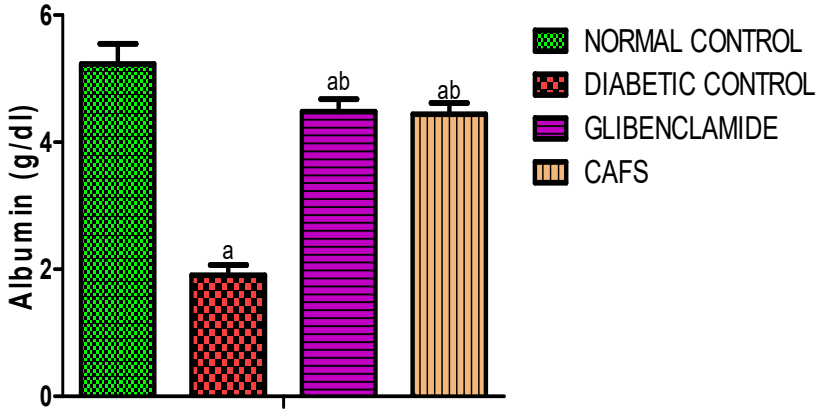

Figure 12: Effect of CAF supplemented diet on Albumin concentration of streptozotocin-induced diabetic rats. **'a'** is significantly different from normal control at ($p < 0.05$), **'b'** is significantly different from diabetic control at ($p < 0.05$).

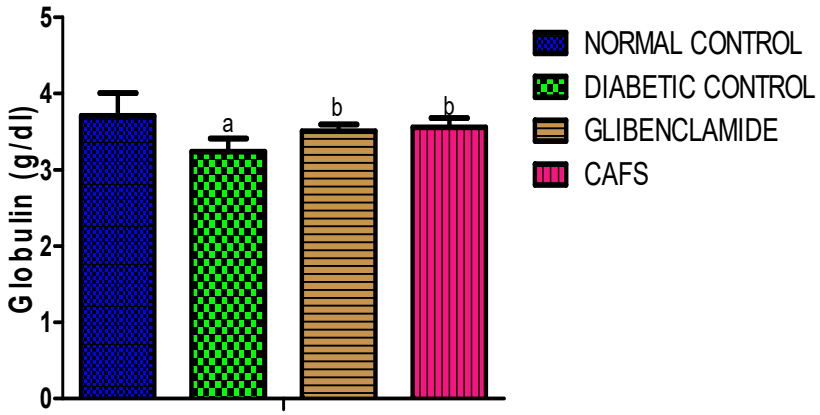

Figure 13: Effect of CAF supplemented diet on Globulin concentration of streptozotocin-induced diabetic rats. **'a'** is significantly different from normal control at ($p < 0.05$), **'b'** is significantly different from diabetic control at ($p < 0.05$).

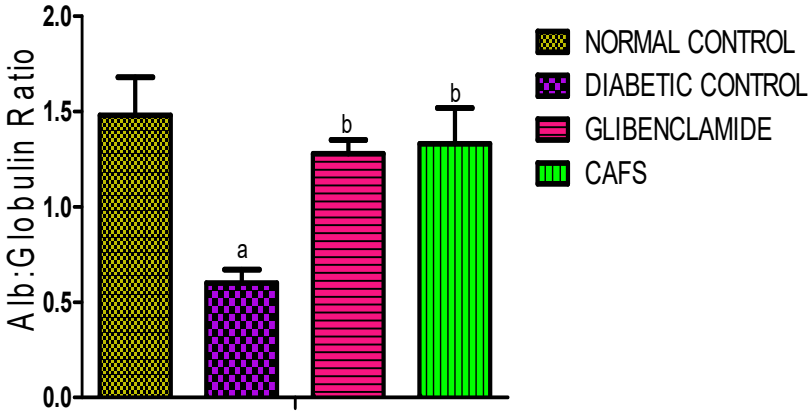

Figure 14: Effect of CAF supplemented diet on Albumin: Globulin ratio of streptozotocin-induced diabetic rats. **'a'** is significantly different from normal control at ($p < 0.05$), **'b'** is significantly different from diabetic control at ($p < 0.05$).

As depicted in Table1, the present study on electrolyte ions concentrations showed that diabetic rats exhibited significant ($p < 0.05$) reduction in plasma K^+, Na^+ and HCO_3^- while increasing the plasma Cl^- compared to rats in the control group. Treatment of diabetic rats with both CAFS supplemented diet and glibenclamide however, produced significant ($p < 0.05$) restorative effects on the altered electrolyte ions concentrations by effecting normalcy in the concentrations.

Table 1: Effects of CAFS on plasma electrolyte ions concentrations (mmol/L) of STZ-induced diabetic rats

Parameters	Normal Control	Diabetic Control	Glibenclamide	CAFS
Potassium (K^+)	4.61 ± 0.09	3.76 ± 0.20[a]	4.69 ± 0.14[b]	4.88 ± 0.27[b]
Sodium (Na^+)	136.78 ± 1.47	111.52 ± 1.24[a]	139.85 ± 0.68[b]	138.71 ± 1.27[b]
Chloride (Cl^-)	138.07 ± 0.78	151.57 ± 1.28[a]	138.45 ± 1.15[b]	139.80 ± 2.49[b]
Bicarbonate (HCO_3^-)	24.14 ± 1.09	19.20 ± 1.15[a]	24.00 ± 1.26[b]	24.22 ± 0.65[b]

Values are expressed as mean± SEM, n= 6. '**a**' represents significant different from normal control at ($p < 0.05$), '**b**' represents significant different from diabetic control at ($p < 0.05$).

Discussion

The liver and kidney function parameters are useful 'biomarkers' for assessment of tissues damages and measurement of activities of various enzymes in the tissues and body fluids and thus, play significant roles in disease investigation and diagnosis (Malomo, 2000). In this study, we observed alterations in the levels of liver and kidney function parameters in STZ-induced diabetic rats and these following the treatment with CAFS supplementation four weeks, these alterations were reversed. Diabetic rats in this study showed elevation of liver maker enzymes (ALT, AST and ALP) activities in the plasma and corroborates those of other authors who also reported increased ALT, AST and ALP activities in STZ-induced diabetic rats (Saeed *et al.,* 2008; Zheng *et al.,* 2010 and Abdel-Moneim *et al.,* 2016). Patients with type 2 diabetes have higher incidence of liver function abnormalities compared to normal individuals (Agarawal, 2015, Ghimire *et al.,* 2018). ALT is a cytoplasmic enzyme found in very high concentration in the liver where it plays a vital role in the metabolism of amino acid (Whitehead *et al.,* 1999). However, during liver assault or damage, ALT leaked from the hepatocytes into the blood circulation and become elevated (Whitehead *et al.,* 1999, Elizabeth and Harris, 2005). This is usually accompanied by a rise in AST activity (Adebayo *et al.,* 2010). ALP is found predominantly in the bile duct of the liver and is an indicator of biliary function, cholestasis and hepatic function (Whitehead *et al.,* 1999, Elizabeth and Harris, 2005). An increase in the activities of AST, ALT and ALP in plasma of diabetic untreated rats could be attributed to the leakage of these enzymes from the liver cytosol into blood stream, giving an indication of hepatic injury and loss of functional integrity (Mirmohammadlu *et al.,* 2015). In diabetes, liver injury may occur because of deleterious effect of hyperglycemia through the inhibition of complex III, generation of reactive oxygen species (ROS) and a change in membrane permeability (Harrison, 2006; Hickman and Macdonald, 2007). This may have a consequential effect on the metabolism and regulation of amino acids in the liver.

The present study showed restoration of elevated levels of AST, ALT and ALP in plasma of diabetic rats treated with CAFS supplementation. Adebayo *et al.* (2011a) reported similar effects on diabetic animals treated with *C. albidum* leaf extracts against CCl_4-induced liver damage. Some phytochemicals such as flavonoids,

triterpenoids, saponins and alkaloids have been linked with hepatic protection. The phytochemical investigation on *C. albidum* fruit skin also showed the presence of flavonoids, phenols, tannins and alkaloids (Ibrahim *et al.,* 2017), suggesting the hepatoprotective role of CAFS against liver injury associated with diabetes. This hepatoprotective property may be due to individual or combined effects of the active secondary metabolites present in CAFS supplemented diet. However, the exact phytochemical (active secondary metabolite) responsible for the hepatoprotective property needs further investigation. The prevention of alteration in the activities of ALT, AST and ALP towards their normal levels may be ascribed to the regeneration of hepatic cells by the active phytochemicals (Ahmad *et al.,* 2000) with hepatoprotective effects in CAFS. This suggests that CAFS may possibly stabilize the plasma membrane and ameliorates hepatic tissue damage (Effiong and Akpan, 2015). Similar to the above trend was the observed changes in plasma AST:ALT ratio. STZ-induced diabetic rats showed an increase in AST: ALT ratio compared to normal control rats. The ratio of AST to ALT has been reported to be a marker of non-alcoholic liver damage occasioned by diabetes or obesity (Shreevastva *et al.,* 2017). Treatment with both CAFS and glibenclamide in the present study however, suppressed these alterations by bringing the values close to the normal values. This result is consistent with the studies of David-Oku *et al.* (2013), who reported a non-dose dependent decrease in AST/ALT ratio of diabetic rats treated with *Gongronema latifolium* crude leaf extract. The present study also agrees with the studies of Shreevastva *et al.* (2017), who reported AST: ALT ratio of less than 2 in most non-alcoholic liver diseases caused by diabetes, obesity or cholesterol.

Furthermore, the present study showed that, diabetic rat is associated with reduction in plasma total protein, albumin, globulin and conjugated bilirubin levels as well as elevation of total bilirubin and unconjugated bilirubin. According to Naganna (1989), an increase in total bilirubin indicates the abnormal liver function, possibly due to higher synthetic function of the liver. Alteration of protein metabolism could occur due to a reduced uptake of amino acids by tissues, an increased rate of muscle proteolysis (Ahmed, 2005) and a reduction in protein synthesis (Tragl and Reaven, 1972).The present study showed restoration of the altered levels of total protein, albumin, globulin, conjugated bilirubin, total bilirubin and unconjugated bilirubin in both CAFS supplementation and glibenclamide treated diabetic rats. Soon and Tan (2002) has also reported a reduction of plasma total protein level in STZ induced diabetic rats treated with *Morinda officinalis.* Low Albumin: Globulin (A/G) ratio has been reported to be associated with diabetes (Mandade and Sreenivas, 2011). A reduced plasma A/G ratio was observed in diabetic rats compared to normal control group. However, this effect was restored following the treatment with CAFS, which could be ascribed to the embedded phytonutrients that work synergistically or elicited a pathway that elevated the plasma A/G ratio. This result is in-line with the earlier report of Sundaram *et al.* (2009), who reported a restoration of the STZ-induced reduction of A/G ratio in diabetic rats treated with various plant extracts.

The kidneys play a pivotal role in the regulation of electrolyte and acid–base balance. Urea and Creatinine are important markers of kidney function. Urea is the predominant nitrogen waste product of protein catabolism; as the rate of proteolysis increases, the production of urea by the liver increases. Creatinine is the major waste product of Creatine metabolism and excreted by the kidneys. In this study, diabetes induces elevation of the plasma urea and Creatinine concentrations. The observed elevation in urea and Creatinine levels signifies the increased proteolysis rate and decreased functional capacity of tubular excretion and suggesting impaired renal function. The observed improvement in the altered levels of urea and Creatinine of diabetic rats treated with CAFS supplementation similar to glibenclamide standard drug treated diabetic rats, suggests a protective role of CAFS on kidneys in diabetes caused kidney dysfunction. In agreement with these results, Latha and Daisy (2011) as well as Tanomand and Najafian (2013) reported the nephroprotective effect of gallic acid isolated from *Terminali abellerica* and cinnamon bark extract respectively against STZ- and gentamicin - induced kidney damage in experimental rats.

Electrolyte imbalances are the early biochemical events responsible for long-term diabetic complications. Electrolyte imbalance occurs in diabetic patients from insulin deficiency, hyperglycemia and hyperketonemia (Kitabchi *et al.,* 2006). In uncontrolled diabetes, kidney function is compromised (Ikpi *et al.,* 2009) with renal loss of electrolytes due to osmotic diuresis (Eteng *et al.,* 2008). This study observed reduction of plasma K^+, Na^+ and HCO_{3-} and on the contrary, elevation of Cl^-concentrations in diabetic rats. The reduction of electrolytes in diabetes may be attributed to dehydration and renal loss of electrolytes by osmotic diuresis (Eteng *et al.,* 2008). However, treatment of diabetic rats with CAFS supplementation reversed the altered levels of plasma K^+, Na^+, $HCO_3^-$and Cl^- to near normal values, indicating the renal protective role of CAFS in diabetic rats. Our previous studies demonstrated that *C. albidum* fruit skin is a potent radical scavenging agent (Ibrahim *et al.,* 2019a), hypolipidemic and hypoglycemic agents (Ibrahim *et al*., 2019b) and thus, suggests its protective role that could ameliorate the development of diabetic complications such as hepatopathy and nephropathy.

Conclusion

Liver and kidney damage are serious complications among patients with DM. This study demonstrated that in STZ-induced diabetic rats, CAFS could be effective in modulating the deleterious effects of chronic diabetes on the liver and kidney of experimental rats by maintaining the liver function enzymes activities and kidney function

markers concentrations toward normal values. CAFS supplementation at 70g/kg feed exhibited similar ameliorative potential with 2.5mg/kg bw glibenclamide (standard drug); hence, suggestive of its protective effects of the liver and kidneys. Thus, CAFS could be considered as therapeutic agent that can ameliorate the progression of diabetic complications. However, further research is required to elucidate the molecular mechanisms of the effects of CAFS.

Authors' contributions

OO designed the study, supervised the laboratory work, and contributed to the critical reading of the manuscript. HOI participated in the design of the study, performed the statistical analysis, drafted the manuscript and participated in final correction of the manuscript. LBM, OK and EOO supervised the animal experiments and the organ collection and participated in final correction of the manuscript. All the authors have read the final manuscript and approved the submission.

Ethical Approval Statement

All procedures and handling of rats during the investigations were reviewed and approved by Babcock University Health Research Ethics Committee (BUHREC approval No. BU/BUHREC029/15).The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH) (1985) and Institute for Laboratory Animal Research (ILAR) (2011).

Conflict of interest

The authors have declared that no conflict of interest exists.

Acknowledgement

The authors gratefully acknowledged Mr Karigidi, K. O. and Mr Olawuni, I. for their contributions and supports and Mr Gisanrin, S. for his technical assistance during the course of this research work.

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