Original Research Article

Antidiabetic and Haematological effects of *Chrysophyllum albidum* supplemented diet on streptozotocin induced diabetic rats

ABSTRACT

Background: The use of food in the management of diseases is an established art of science. It is essentially important in the management of chronic diseases such as diabetes mellitus where cure is not certain.

Objective: This study investigated the anti-diabetic and heamatological effects of *Chrysophyllum albidum* fruit-skin (CAFS) supplemented diet on streptozotocin induced diabetic rats.

Methods: Diabetes mellitus was induced by a single intraperitoneal injection of 50 mg/kg streptozotocin (STZ) and 70g/kg CAFS supplemented diet was used on STZ-induced diabetic rats to test its antidiabetic efficacy with some biochemical parameters and histological evaluation of liver and pancreatic tissues for a treatment period of twenty-eight days.

Results: The diabetic untreated rats exhibited hyperglycaemia accompanied with increases in glycosylated haemoglobin, plasma and liver lipid profile except high density lipoprotein-cholesterol (HDL-c) and white blood cell count while decreasing the body weight, insulin, hepatic glycogen and red blood cell levels. CAFS was significantly ($p<0.05$) effective in inhibiting hyperglycemia by 68%, decreased glycosylated haemoglobin by 20%, plasma and liver lipid profile except HDL-c and white blood cell count but increased the body weight by 17%, insulin, hepatic glycogen and red blood cell levels in comparison with diabetic untreated group. The results were comparable to glibenclamide (standard-drug). Histopathological studies on liver and pancreas of CAFS-treated rats showed regenerative effects.

Conclusion: This study has indicated that CAFS possesses antihyperglycemic, antihyperlipidemic and ameliorative effect on diabetic induced abnormalities in haematological parameters, β-cell and liver tissue. The findings suggest that CAFS may be used as therapeutic adjunct in the management of diabetes.

Keywords: antidiabetic; haematological; *Chrysophyllum albidum*; fruit-skin; streptozotocin.

1. Introduction

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion or utilization. The hallmark of diabetes mellitus is polyuria, polydipsia and polyphagia. Chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and eventually the failure of organs, especially the eyes, kidneys,

nerves, heart and blood vessels [1]. Among the complications associated with diabetes, include hyperlipidaemia [2, 3] and haematological abnormalities [4].

The global prevalence of diabetes is estimated to increase from 4% in 1995 to 5.4% by the year 2025 [5]. The WHO [6] report estimated that 1.7 million people in Nigeria had diabetes with the projection that the number will triple by 2030. People with diabetes is increasing due to population growth, aging, consumption of energy rich diet and increasing prevalence of obesity and physical inactivity [7]. Despite the great efforts made to better understand and manage this disease, serious problems such as nephropathy, retinopathy and lower extremity amputation continue to affect patients, while diabetes-related mortality continue to rise [8]. Currently, there are different groups of oral hypoglycemic agents for clinical use and having characteristic profiles of side effects [9]. Management of diabetes without any side effects is still a challenge to the medical system. This has led to an increase in demand for natural food products with anti-diabetic activity and lesser side effects.

Chrysophyllum albidum (*C. albidum*), also known as African star apple, is primarily a forest tree species (Figure 1a) with its occurrence in the Central, East and West Africa regions. It belongs to Sapotaceae family. In Nigeria, it is widely grown in the South Western part and locally called “agbalumo”. The fruit of *C. albidum* (Figure 1b) is traditionally used for nutritional purposes and to relief gastrointestinal tract disturbances [10]. The stem bark is used for the treatment of malaria and yellow fever, while the leaf is used as an emollient and for the treatment of skin eruption, stomach-ache and diarrhea [11]. The seeds, roots and leaves extracts are used to arrest bleeding from fresh wounds, inhibit microbial growth and enhance wound healing process [12]. The root and stem bark extracts has been reported for the anti-fertility [13] and antimicrobial [14] effects.The anti-hyperglycemic and hypolipidemic effect of ethanol extract of the seed cotyledon [15] and leaf [16] have been evaluated. Adebayo *et al*. [17] and Omotosho *et al*. [18] reported the antioxidant effects of the leaf and fruit juice respectively. Our earlier studies demonstrated that *C. albidum* fruit skin contains heterogeneous phytoconstituents with potential hypoglycemic effect [19]. In another studies, the fruit-skin methanolic extract of *C. albidum* exhibited comparable radical scavenging effects and inhibitory effect on lipid peroxidation, α-amylase and α-glucosidase activities with the standards and suggest that *C. albidum* fruit skin could be an excellent candidate for future studies on diabetes [20]. In line with these findings and vast applications of this plant in the literature, this study focused on the anti-diabetic and

haematological effects of *Chrysophyllum albidum* fruit skin (CAFS) supplemented diet (Table 1) in streptozotocin induced diabetic rats.

Glibenclamide, being the most widely used oral hypoglycemic agent and which functions by stimulating insulin secretion leading to increased responsiveness of ß-cells to both glucose and non-glucose secretagogues and resulting in more insulin being released to reduce blood glucose concentrations [21], was chosen as a positive control in the study.

Figure 1: *Chrysophyllum albidum* tree (a) and fruits (b)
Source: Orwa *et al*. [22]

2. Materials and Methods

2.1 Plant materials

The fresh fruits of *C. albidum* were purchased at Moniya market, Akinyele Local Government Area of Oyo State, South-Western Nigeria. The fruit was identified and authenticated in the herbarium unit of Botany Department, University of Ibadan, Oyo State, Nigeria where a voucher specimen was deposited with the voucher registration No. UIH/20I6/22502.

2.2 Preparation of plant materials

The fresh riped fruit of *C. albidum* was separated, washed, weighed and its seed-shell pericarp, fruit- pulp and fruit- skin (samples) removed and cut into small pieces. The samples were lyophilized for 54 h using Lyophilizer Millorock Bench-Top Freeze Dryer, Germany. Lyophilized samples were stored at -20ºC until further use.

2.3 Experimental design

Feed formulation was made based on the recommendations of National Academy of Sciences [23] on nutrients requirement for the laboratory rats for growth and maintenance (Table 1). Thirty-two male albino rats weighing 160 ± 20 g were purchased from the Animal Unit of Babcock University, Ilishan-Remo, Ogun State, Nigeria. Rats were housed in cages under a controlled light cycle (12 h light / 12 h dark) and randomly divided into four groups. The rats were acclimatized for a period of two weeks, placed on commercially available feed and water administered *ad libitum* during the acclimatization period.

2.4 Animal care and approval

The care and handling of the rats as well as experimental protocols were duly approved by Babcock University Health Research Ethics Committee (BUHREC) with the BUHREC approval No. BU/BUHREC029/15 in accordance with the Institute for Laboratory Animal Research Guides for the Care and Use of Laboratory Animals [24, 25].

2.5 Induction of diabetes mellitus

Diabetes mellitus (DM) was induced by a single intraperitoneal injection of 50 mg/ kg of streptozotocin (STZ) (Sigma chemical Co) as described by Thirumalai *et al*. [26] and Prasath *et al*. [27] with little modifications. The freshly prepared STZ in 0.1M citrate buffer (pH 4.5) was administered into groups 2-4 fasted (12 h) rats, while group 1 (non-diabetic rats) was injected with citrate buffer alone through the same route. Rats were provided with 5% glucose solution after 6 h of STZ administration for the next 12 h to overcome STZ-induced hypoglycemia. Seventy-two hours (72 h) after STZ administration, blood samples were taken by tail vein puncture and fasting blood glucose levels ≥ 250 mg/dl were considered diabetic and used in the study with Accu-chek glucometer (Roche Diagnostics-GmbH, Germany).

2.6 Experimental protocol

Each of the four groups consists of eight rats:

Group 1: *Normal control group:* Non-diabetic rats, received 1 ml normal saline given orally once a day by gastric tube for four weeks, fed normal control (NC) (basal) diet and served as normal control group.

Group 2: *Diabetic control group*: Diabetic rats, received 1 ml normal saline given orally once a day by gastric tube for four weeks, fed NC (basal) diet and served as diabetic-untreated group.

Group 3: Diabetic treated group with glibenclamide: Diabetic rats, received 2.5mg/kg b.w. of glibenclamide in 1 ml normal saline given orally once a day by gastric tube for four weeks, fed NC (basal) diet and served as glibenclamide-treated group.

Group 4*:* Diabetic treated group with CAFS: Diabetic rats, received 1 ml normal saline given orally once a day by gastric tube for four weeks, fed 70g/kg of freeze-dried *Chrysophyllum albidum* fruit skin (CAFS) supplemented diet and served as CAFS-treated (test) group.

Body weight, food intake and fasting blood glucose levels were measured on weekly intervals. Other biochemical parameters such as serum insulin, glycosylated haemoglobin, hepatic glycogen, plasma and liver lipid profile contents and haematological indices were evaluated at the end of study. The rats were fasted overnight and sacrificed by cervical dislocation after 4 weeks.

Table 1. Chemical composition of normal control (NC)- and CAFS supplemented diets

Components	NC (basal) diet (g/kg)	CAFS diet (g/kg)
Carbohydrate (Corn meal)	480	480
Protein (Casein)	200	200

Unsaturated fat (Soya bean oil)	70	70
Fiber (Rice bran)	100	30
Fiber (CAFS)	-	70
Sucrose	100	100
#Vitamin mix	10	10
*Mineral mix	35	35
Methionine	3.5	3.5
Lysine	1.5	1.5
Total	**1000**	**1000**
Calculated Gross Energy (kJ/1000g Feed)	15665	15665

Gross energy (kJ per 100 g dry matter) = (crude protein × 16.7) + (crude lipid × 37.7) + (carbohydrate × 16.7)

#-One kilogram of vitamin- mix contains: 12,000 IU vitamin A, 2,400 IU vitamin D3, 20 mg vitamin E, 4 mg vitamin K3, 3 mg vitamin B1, 7 mg vitamin B2, 25 mg niacin (vitamin B3), 10 mg pantothenic acid (vitamin B5), 5 mg vitamin B6, 15 µg vitamin B12, 50 µg biotin, 1 mg folic acid and 50 mg vitamin C.

*-One kilogram of mineral- mix contains: 100 mg Mn, 60 mg Fe, 60 mg Zn, 5 mg Cu, 2 mg I, 500 µg Co and 150 µg Se. The protein content of CAFS was taken into consideration in the formulation of CAFS diet. The feed was pelletized to make it appealing to the rats.

NC diet: Diet formulation without CAFS (basal diet); CAFS diet: Diet formulation with 70g/kg- freeze-dried *Chrysophyllum albidum* fruit- skin.

2.7 Oral glucose tolerance test (OGTT)

On the 25th day of the treatment, oral glucose tolerance test was performed on overnight fasted rats. Each rat was given a single dose of 2 g/kg of glucose dissolved in distilled water (40 % w/v) by oral gavage. Blood samples were collected from the tail vein at 0, 30, 60, 90 and 120 min after the oral glucose load. The blood glucose concentration was determined using Accu-chek glucometer (Roche Diagnostics-GmbH, Germany) and expressed as mg/dl.

2.8 Physiological parameters

Feed intake and body weight were monitored on weekly intervals; urine glucose was monitored on 0^{th}, 2^{nd} and 28^{th} day of treatment using urine color-matching test strips (Uristix from Bayer Diagnostics) and relative organ weights was also estimated using the formula:

$$\text{Relative organ weight (g/100g)} = \frac{\text{Absolute organ weight}}{\text{Final body weight}} \times 100 \quad (1)$$

2.9 Biochemical estimations

Fasting blood glucose was estimated by Accu-chek glucometer and Glycosylated hemoglobin (HbA1c) by TC Matrix analyzer for glycosylated haemoglobin Reagent kit from

Teco Diagnostics, USA according to the manufacturer's instructions. Hepatic glycogen content was determined by the method of Ong and Khoo [28] while serum insulin level was determined using rat insulin ultral-sensitive ELISA kit (product code: 2425-300), according to the manufacturer's instructions. Plasma and liver tissue homogenates were evaluated for lipid profile [total cholesterol (TC), triglyceride (TG), and high-density lipoprotein-cholesterol (HDL-c) using Randox diagnostic kits [29]. Low-density lipoprotein-cholesterol (LDL-c) and very low-density lipoprotein-cholesterol (VLDL-c) concentrations were calculated from the measurement using the formula of Friedwald *et al.* [30]. Values were expressed as mg/dl.

2.10 Heamatological estimation

Blood was collected by cardiac puncture for heamatological indices estimations using an automated haematologic analyzer (Swelab Alfa 3-part haematology analyzer, Boule Medicals) according to the methods described by Baker *et al.* [31] and Cheesbrough [32].

2.11 Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA) followed by least significant difference (LSD) post Hoc-test. Differences were considered to be significant when $P < 0.05$, $P < 0.01$ or $P < 0.001$ and data expressed as mean ± SEM of six determinations (n=6).

3. Results

3.1 Average feed intake, body weight and urine sugar

Data in Tables 2 & 3 revealed that CAFS- and glibenclamide treated diabetic rats showed significant decrease in the levels of feed intake and a significant increase in the body weight gain from the second week of treatment ($p < 0.05$) till the end of treatment ($p < 0.001$) when compared to diabetc untreated (DC) rats. The increased urine glucose concentration in all STZ-induced diabetic rats (groups 2-4) on day second of treatment was reversed at twenty-eighth day of treatment in CAFS- and glibenclamide treated diabetic rats. CAFS- treated diabetic rats showed only traces of glucose in their urine while no trace of glucose concentration was observed in the urine of glibenclamide treated diabetic rats (Table 4).

Table 2. Feed intake (g) of normal control and streptozotocin induced diabetic rats fed with or without CAFS diet

Time (wk)	Normal control	Diabetic - untreated	Glibenclamide- treated	CAFS- untreated
Week One	13.89±0.29	14.52±0.29$^{\#}$	13.66±0.17*	13.71±0.20*
Week Two	14.63±0.27	17.05±0.31^{c}	14.49±0.22^{d}	15.29±0.37^{b}
Week Three	15.79±0.22	20.90±0.33^{e}	15.52±0.30^{f}	17.26±0.48^{a,d}
Week Four	16.84±0.27	24.21±0.15^{e}	16.42±0.15^{f}	19.18±0.37^{c,f}

Values are expressed as x ± SEM (n=6). Levels of significance: Compared with normal control (NC): 'a' $p<0.05$), 'c' $p<0.01$), 'e' $p<0.001$), '#' $p> 0.05$ –No significant difference; Compared with diabetic untreated (DC): 'b' $p<0.05$), 'd' $p<0.01$, 'f' $p<0.001$), * $p> 0.05$ – No significant difference.

Table 3. Body weight (g) of normal control and streptozotocin induced diabetic rats fed with or without CAFS diet

Groups/Time	Normal control	Diabetic-untreated	Glibenclamide- treated	CAFS- untreated
Day Zero	209.22±3.36	212.22±2.44$^{\#}$	211.11±2.73*	210.27±2.25*
Day Three	225.27±1.46	217.88±2.63$^{\#}$	218.80±2.11*	216.94±3.03*
Week One	227.64±2.36	216.15±3.56$^{\#}$	219.94±3.07*	215.98±3.47*
Week Two	236.27±1.77	209.27±3.92^{e}	224.50±3.23^{a,d}	222.50±3.93^{b,c}
Week Three	240.92±1.55	200.66±3.46^{e}	228.67±3.58^{a,f}	224.30±5.36^{c,f}
Week Four	247.97±1.16	194.11±3.11^{e}	233.67±1.80^{a,f}	226.88±6.24^{c,f}

Values are expressed as x ± SEM (n=6). Levels of significance: Compared with NC: 'a' $p<0.05$), 'c' $p<0.01$), 'e' $p<0.001$), '#' $p> 0.05$ –No significant difference; Compared with DC: 'b' $p<0.05$), 'd' $p<0.01$, 'f' $p<0.001$), * $p> 0.05$ – No significant difference.

Table 4. Urine sugar screening of normal control and streptozotocin induced diabetic rats

Time (Day)	Normal control	Diabetic - untreated	Glibenclamide- treated	CAFS- untreated
0^{th} day (pre-STZ-induction)	-	-	-	-
2^{nd} day of treatment	-	+++	+++	+++
28^{th} day of treatment	-	+++	-	+

+++ =Highly concentrated; + = Traces; - =Absent.
Normal Control Rats: Normal rats fed NC (basal) diet; Diabetic untreated: STZ-Induced and untreated rats, fed NC (basal) diet;
Glibenclamide: STZ-induced rats and treated with standard drug-Glibenclamide (2.5mg/kg bw/day/rat) by oral gavage, fed NC (basal) diet;
CAFS: STZ-induced rats and treated with 70g/kg supplemented freeze-dried *Chrysophyllum albidum* fruit-skin diet.

3.2 Relative organ weights

Diabetic untreated rats exhibited significant ($p < 0.001$) increase in the relative weights of liver and kidney while the relative weight of pancreas was significantly ($p < 0.001$) decreased when compared to normal control (Figure 2). Four weeks treatment with CAFS diet/ glibenclamide was effectively associated with reversed alteration in liver, kidney and pancreas weights while no significant changes ($p > 0.05$) was observed in the relative heart weight of diabetic rats treated and untreated, as compared to normal control.

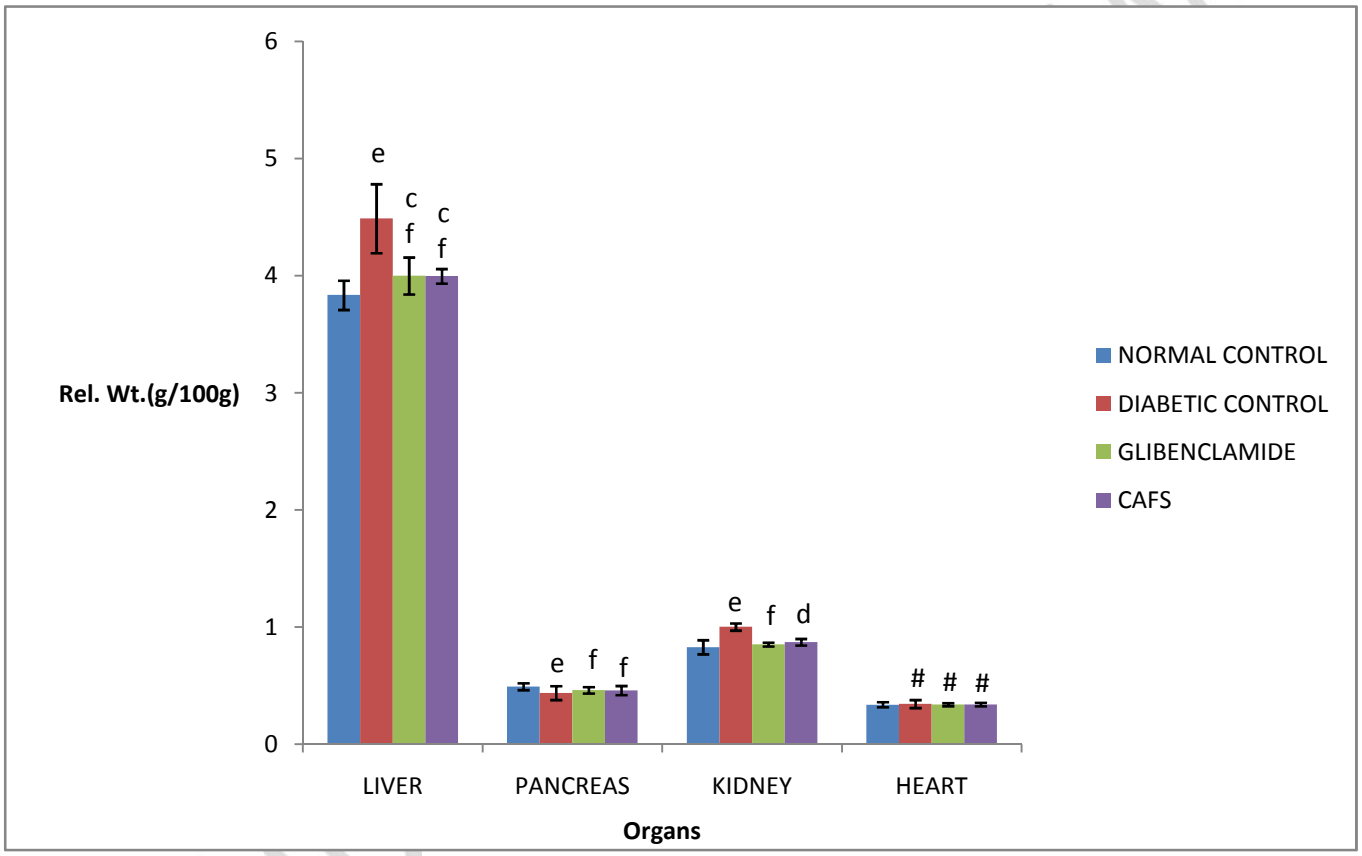

Figure 2: Relative organs weight of normal control and STZ-induced diabetic rats fed with or without CAFS diet
c is significantly different from normal control at ($p < 0.01$)
d is significantly different from diabetic control (diabetic untreated) ($p < 0.01$)
e is significantly different from normal control at ($p < 0.001$)
f is significantly different from diabetic control ($p < 0.001$)
# indicates No significant difference ($p > 0.05$) from normal control and diabetic control (untreated)

3.3 Oral glucose tolerance test and fasting blood glucose level

As shown in Figure 3, oral glucose tolerance test (OGTT) of diabetc untreated rats showed a pronounced elevation of blood glucose level from (375.81±1.70 mg/dl to 435.12 ±10.22 mg/dl) after 30 minutes of oral administration of glucose (2g/kg bw). This elevation reached its peak value (441.53 ±1.46 mg/dl) at 60 minutes of glucose intake and began to

decrease slowly during the next 60 minutes to reach 419.95±2.05mg/dl after 2 hours of glucose administration. CAFS treated diabetic rats also showed increase in blood glucose level at 25th day of treatment from 130.16±1.32 mg/dl to 289.11 ±4.69 mg/dl) after 30 minutes of glucose intake. This increase reached its peak value (318.30 ±2.95 mg/dl) at 60 minutes of glucose intake and began to decline rapidly during the next 60 minutes to reach 123.24±3.17mg/dl after 2 hours of glucose administration. The result was comparable with glibenclamide treated values (313.51±5.79 mg/dl and 111.58±3.49mg/dl) at 60 and 120minutes of glucose load respectively.

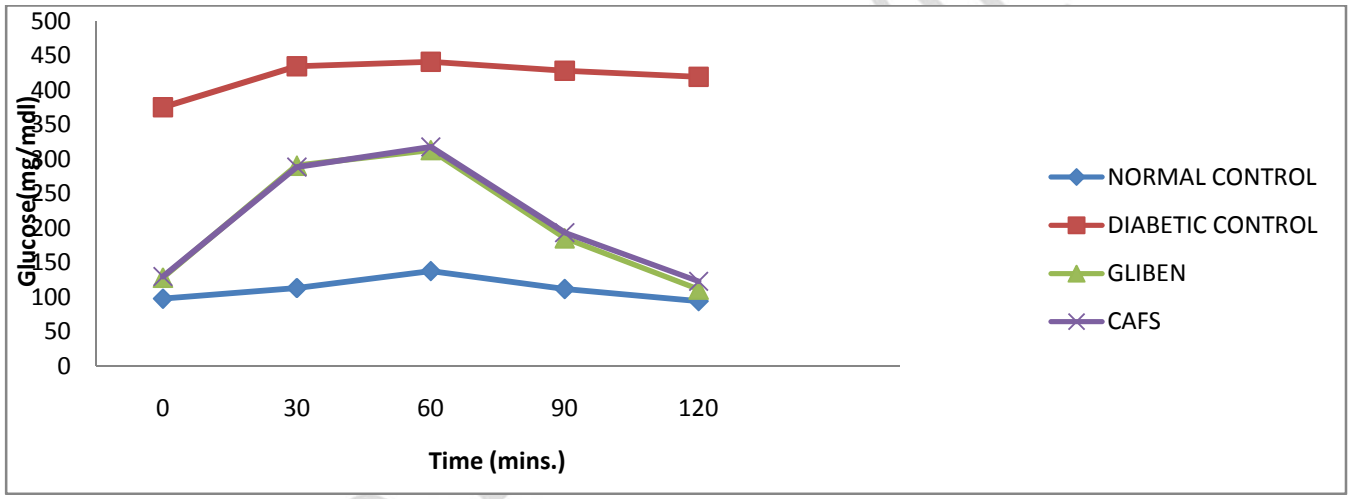

Figure 3. Changes in oral glucose tolerance test of STZ-induced diabetic rats fed with or without CAFS diet

GLIBEN: Glibenclamide.

CAFS treated diabetic rats showed significant ($p < 0.001$) reduction in fasting blood glucose level throughout the 28 days of treatment (Table 5) as compared to diabetc untreated rats. The result was comparable to normal control and glibenclamide treated rats.

Table 5. Fasting blood glucose (mg/dl) of normal control and streptozotocin induced diabetic rats fed with or without CAFS diet

Groups/Time (day/wk)	Normal control	Diabetic - untreated	Glibenclamide-treated	CAFS- untreated
Day 0	94.96±2.76	94.91±2.07[#]	95.57±1.28*	94.61±2.29*
Day 3	95.96±1.76	289.36±6.23[e]	290.23±4.87[e]	289.11±5.08[e]
Week One	98.19±1.71	339.50±3.22[e]	295.51±6.35[d,e]	301.99±4.66[d,e]
Week Two	95.01±1.93	370.50±2.84[e]	230.69±4.72[e,f]	253.35±8.06[e,f]
Week Three	96.03±1.48	374.75±5.25[e]	176.37±5.54[e,f]	167.25±3.17[e,f]
Week Four	95.47±1.12	377.49±5.57[e]	100.82±8.38[f]	122.66±3.77[c,f]

Values are expressed as x ± SEM (n=6). Levels of significance: Compared with NC: 'c' p<0.01), 'e' p<0.001), '#' p> 0.05 –No significant difference; Compared with DC:' d'p<0.01, 'f' p<0.001), * p> 0.05 – No significant difference.

3.4 Glycogen, glycosylated heamoglobin and insulin

The levels of hepatic glycogen, glycosylated haemoglobin and serum insulin in normal and diabetic rats are depicted in Table 6. As compared to the normal control rats, diabetic condition caused significant decrease ($p < 0.001$) in the levels of hepatic glycogen and serum insulin while increasing the level of glycosylated heamoglobin. Four weeks treatment with CAFS diet, significantly ($p < 0.001$) increased the levels of hepatic glycogen and serum insulin by 58% and 49% respectively as compared to diabetc untreated while glycosylated heamoglobin was significantly ($p < 0.001$) decreased by 20%. The result was comparable with glibenclamide treated rats.

Table 6. Hepatic glycogen, glycosylated haemoglobin and serum insulin of normal control and STZ-induced diabetic rats fed with or without CAFS diet

Groups / Parameters	Normal control	Diabetic-untreated	Glibenclamide- treated	CAFS- untreated
Hepatic Glycogen (mg/g wet tissue)	12.68±1.27	4.06±0.98[e]	11.43±0.84[f]	9.63±0.45[a,f]
Glycosylated Heamoglobin (%)	5.10±0.04	6.83±0.18[e]	5.40±0.06[e,f]	5.45±0.16[e,f]
Serum Insulin (μU/ml)	10.55±0.32	4.97±0.16[e]	7.68±0.39[e,f]	7.40±0.49[e,f]

Values are expressed as x ± SEM (n=6). Levels of significance: Compared with NC: 'a' p<0.05, 'e' p<0.001; Compared with DC: 'f' p<0.001).

3.5 Histopathological evaluation

3.5.1 Microscopic Plates: Presents the histological sections of the liver tissues of normal control group and STZ-diabetic treated and untreated rat groups.

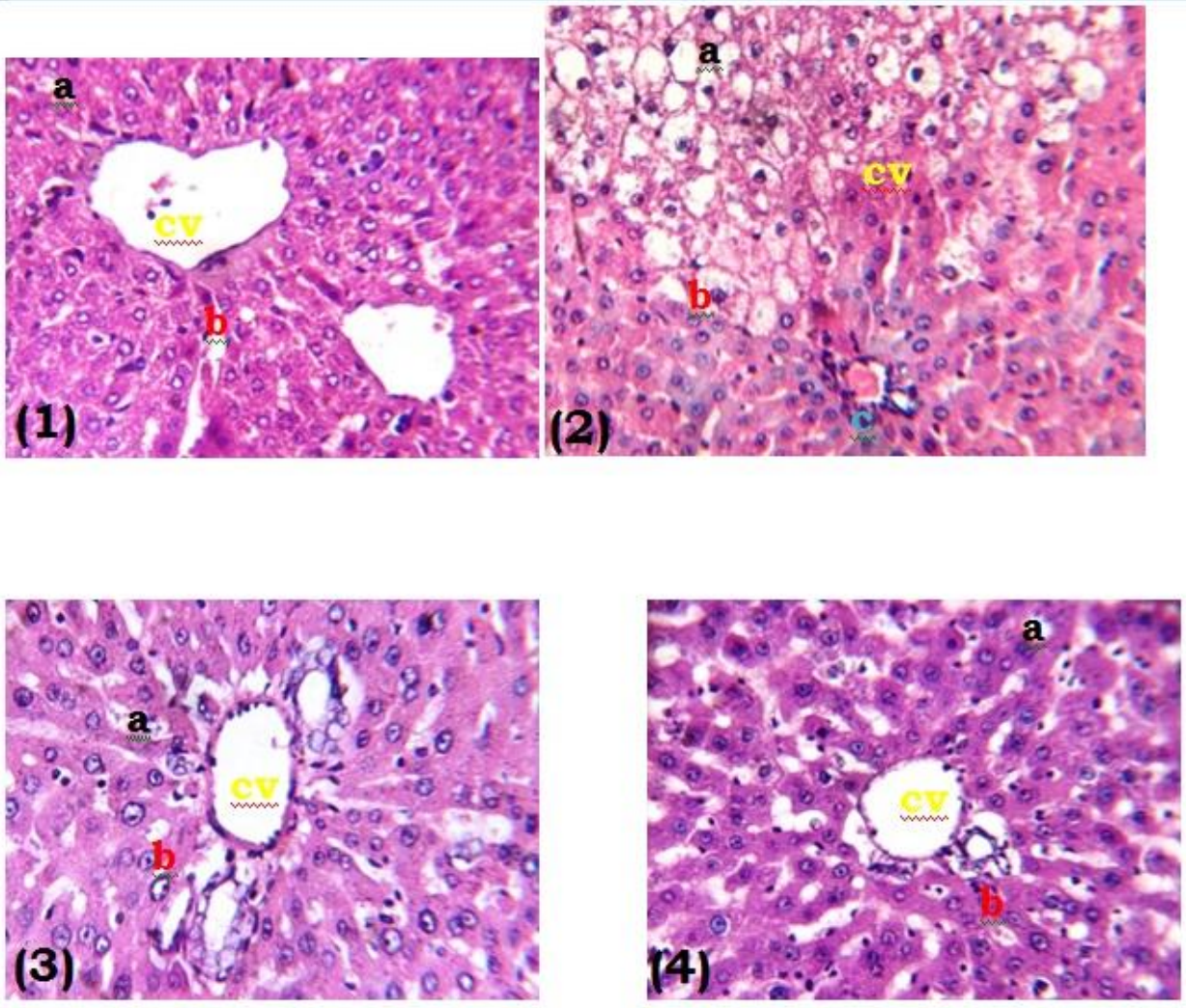

Plate 1: Photomicrograph of hepatic tissues (H&E x 400)

Normal control (1) : showing normal morphology of liver tissue portraying normal central vein (cv) with radiating cords of hepatocytes, normal macrovesicular (a) and normal microvesicular steatosis (b)**; Diabetic untreated (2):** showing loss of normal architecture of liver tissue with destructed central vein (cv); disorganized macrovesicular (a), microvesicular steatosis (b) and a focal area of inflammation (c); **Diabetic treated with glibenclamide (3):** showing structural restoration of the central vein (cv) with radiating hepatocytes and portal triad (b) **and Diabetic treated with CAFS-supplemented diet (4):** showing structural improvement with dilation (a) and mild congestion of central vein (cv), normal architecture of portal triad (b) and hepatocytes.

3.5.2 Microscopic Plates: Presents the histological sections of the pancreatic tissues of normal control group and STZ-diabetic treated and untreated rat groups.

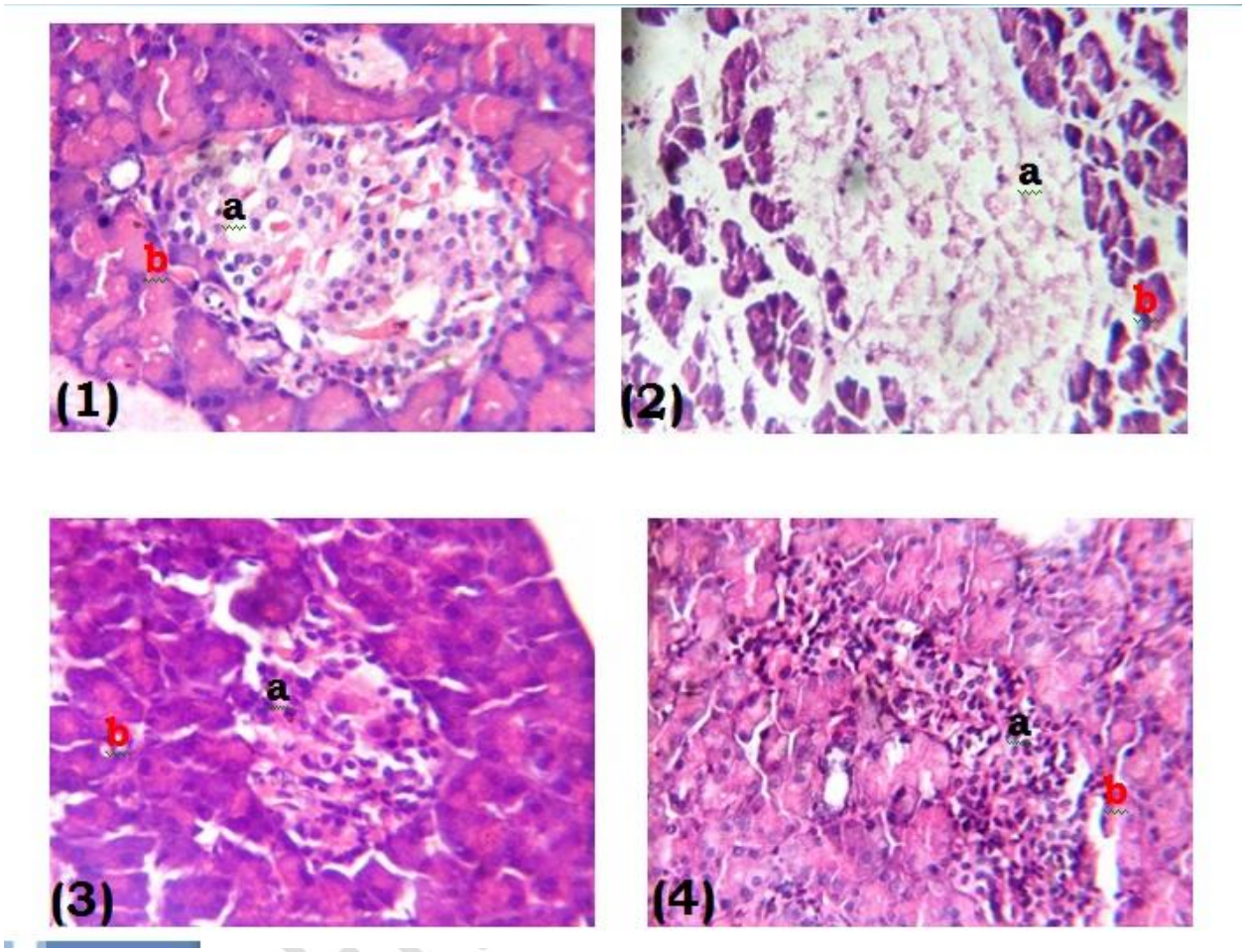

Plate 2: Photomicrograph of Pancreatic Tissues (H&E x 400)

Normal Control (1): showing normal Islets of Langerhan (a) and pancreatic acini (b). No lesion seen; **Diabetic untreated (2):** showing severe necrosis, cell reduction of the Islet of Langerhan (a) and pancreatic acini (b); **Diabetic treated with Glibenclamide (3):** showing mild inflammation of the interstitial, restoration of Islet Langerhan (a) and normal pancreatic acini (b) and **Diabetic treated with CAFS-supplemented diet (4):** showing structural improvement with mild reduction in the number of islet of Langerhans, mild congestion in the interstitial (a) and normal pancreatic acini (b).

3.6 Lipid profiles of liver and plasma

The levels of liver and plasma lipid profile in normal and diabetic rats are shown in Tables 7 and 8 respectively. The levels of liver and plasma lipid profile except HDL-c were significantly elevated ($p < 0.001$) in the diabetc untreated rats as compared to normal control rats. Treatment of diabetic rats with CAFS diet resulted in significant decrease ($p < 0.001$) of liver and plasma

lipid profiles except HDL-c when compared with the diabetic untreated. The levels of liver and plasma coronary risk (TC: HDL-c) and atherogenic (LDL-c: HDL-c) indices were also decreased significantly relative to diabetic untreated. The result was comparable with glibenclamide treated rats.

Table 7. Liver lipid profile of normal control and STZ-induced diabetic rats fed with or without CAFS diet

Groups/ Parameters	Normal control	Diabetic- untreated	Glibenclamide- treated	CAFS- untreated
TC	52.75±3.36	110.189±2.53^{e}	68.63±0.91^{e,f}	68.60±1.31^{e,f}
TG	42.43±2.83	99.23±3.42^{e}	38.91±0.85^{f}	40.86±1.96^{f}
HDL	30.54±0.65	23.12±0.15^{e}	34.77±0.19^{e,f}	34.20±0.26^{e,f}
LDL	13.73±2.89	67.22±2.31^{e}	26.08±1.00^{e,f}	26.23±1.18^{e,f}
VLDL	8.49±0.57	19.85±0.68^{e}	7.78±0.17^{f}	8.17±0.39^{f}
TC:HDL	1.72±0.07	4.77±0.11^{e}	1.97±0.03^{a,f}	2.00±0.03^{a,f}
LDL:HDL	0.44±0.08	2.91±0.10^{e}	0.75±0.03^{c,f}	0.76±0.03^{c,f}

Values are expressed as x ± SEM (n=6). Levels of significance: Compared with NC: 'a' p<0.05), 'c' p<0.01), 'e' p<0.001); Compared with DC: 'f' p<0.001). TC: total cholesterol, TG: triglyceride, HDL: high-density lipoprotein, LDL: low-density lipoprotein, VLDL: very low-density lipoprotein.

Table 8. Plasma lipid profile of normal control and STZ-induced diabetic rats fed with or without CAFS diet

Groups/ Parameters	Normal control	Diabetic- untreated	Glibenclamide-treated	CAFS- untreated
TC	70.41±1.15	157.84±1.86^{e}	70.05±0.88^{f}	70.54±1.59^{f}
TG	41.73±2.24	158.02±1.62^{e}	110.01±2.49^{e,f}	80.26±2.58^{e,f}
HDL	30.63±0.71	9.04±0.18^{e}	14.15±0.30^{e,f}	20.43±0.18^{e,f}
LDL	31.44±0.99	117.19±1.81^{e}	33.90±1.00^{f}	34.06±1.28^{f}
VLDL	8.35±0.45	31.60±0.32^{e}	22.00±0.50^{e,f}	16.05±0.52^{e,f}
TC:HDL	2.31±0.71	17.48±0.32^{e}	4.96±0.14^{c,f}	3.46±0.09^{a,f}
LDL:HDL	1.03±0.05	12.98±0.24^{e}	2.40±0.10^{c,f}	1.67±0.68^{a,f}

Values are expressed as x ± SEM (n=6). Levels of significance: Compared with NC: 'a' p<0.05), 'c' p<0.01), e' p<0.001; Compared with DC 'f' p<0.001. TC: total cholesterol, TG: triglyceride, HDL: high- density lipoprotein, LDL: low- density lipoprotein, VLDL: very low- density lipoprotein.

3.7 Haematological parameters

The heamatological parameters of control and experimental animals are presented in Table 9. Diabetic untreated rats significantly lowered red blood cell (RBC) ($p < 0.05$), haemoglobin (Hb), mean corpuscular haemoglobin concentration (MCHC) and platelet counts ($p < 0.01$) levels as well as packed cell volume (PCV) ($p < 0.001$) level when compared with the normal

control group. On the other hand, significant elevation of lymphocyte and mid-cells (basophil + eosinophil + monocyte) ($p < 0.05$) levels as well as white blood cell (WBC) and neutrophil ($p < 0.01$) levels were observed while no significant ($p>0.05$) changes was observed in MCH level of diabetic untreated animals as compared to normal control group. Four weeks treatment with CAFS however, significantly raised the lowered RBC ($p < 0.05$); Hb, PCV and platelet counts ($p < 0.01$) while significantly ($p < 0.05$) lowered the elevated WBC count, neutrophil and lymphocyte levels. The results compared favourably with that of glibenclamide, a standard drug.

Table 9. Haematological indices of normal control and STZ-induced diabetic rats fed with or without CAFS diet

Groups/ Parameters	Normal control	Diabetic- untreated	Glibenclamide- treated	CAFS- untreated
Heamoglobin (g/dl)	14.22± 0.75	8.37± 1.74^{c}	13.73± 0.78^{d}	13.37± 1.13^{d}
Heamatocrit (%)	40.85± 3.41	26.50± 4.89^{e}	42.92± 1.70^{f}	41.52± 3.23^{d}
RBC (x 10^{12}/L)	7.21± 0.66	4.28± 0.71^{a}	7.77± 0.20^{b}	6.61± 0.55^{b}
MCV (fl)	57.37± 2.82	69.19± 6.40^{a}	55.15± 0.94^{b}	63.09± 1.22$^{\#*}$
MCH (pg)	20.29± 1.45	21.00± 1.32$^{\#}$	17.70± 0.92^{b}	20.25± 0.47$^{\#*}$
MCHC (g/dl)	35.29± 1.39	30.75± 0.93^{c}	32.16± 1.80$^{\#*}$	32.10± 0.32$^{\#*}$
Plt.C (x 10^{9}/L)	455.00± 52.43	349.00± 58.10^{c}	450.17± 20.24^{d}	448.33± 35.32^{d}
WBC (x 10^{9}/L)	8.17± 1.17	12.77± 4.01^{c}	9.58± 1.47^{b}	9.83± 2.09^{b}
NEU (%)	12.73± 1.69	20.47± 2.79^{c}	15.65± 3.29^{b}	15.60± 0.92^{b}
LYM (%)	69.52± 3.69	77.78± 3.04^{a}	71.40± 6.37^{b}	71.95± 2.98^{b}
Midcell (%)	9.17± 1.70	14.00± 1.84^{a}	11.83± 2.85$^{\#*}$	11.33± 0.95$^{\#*}$

Values are expressed as x ± SEM (n=6). Levels of significance: Compared with NC: 'a' p<0.05), 'c' p<0.01), e' p<0.001, '#' p> 0.05 –No significant difference; Compared with DC: 'b' p<0.05), 'd'p<0.01, 'f' p<0.001, * p> 0.05 – No significant difference.
RBC- Red blood cell count; MCV- Mean corpuscular volume; MCH-Mean corpuscular haemoglobin; MCHC-Mean corpuscular haemoglobin concentration; Plt. C-Platelet count; WBC- White blood cell count; NEU-Neutrophil; LYM-Lymphocyte; Midcell-(Basophil+ Eosinophil+ Monocyte).

4. Discussion

Diabetes mellitus is a chronic metabolic disease characterized by hyperglycemia and disturbances in carbohydrate, fat and protein metabolism. In addition to hyperglycemia, hyperlipidemia is involved in the development of the microvascular complications of diabetes, all of which are the major causes of morbidity and mortality [2]. The link between chronic diseases and anemia is well characterized [33]. The occurrence of anaemia in diabetes mellitus has been linked to the increase in non-enzymatic glycosylation of RBC membrane proteins, which correlates with hyperglycemia [34, 27].

In the present study, the effect of *Chrysophyllum albidum* fruit-skin (CAFS) supplemented diet was investigated in streptozotocin-induced diabetic male albino rats. Body weight, feed intake and fasting blood glucose of control and experimental rats were evaluated on weekly basis while other biochemical parameters such as serum insulin, glycosylated haemoglobin, hepatic glycogen, plasma and liver lipid profile contents and haematological indices were assessed at the end of treatment (four weeks). Streptozotocin (STZ) exerts its diabetogenic effect by selectively destroying the pancreatic β-cells, which causes less active pancreatic cells and produces diabetes mellitus [35]. From the results of the present study, the reduction in serum insulin level of diabetic untreated rats could be attributed to pancreatic β-cells damage induced by STZ- diabetes as indicated in this study. On the other hand, the results obtained for plasma glucose concentration of diabetic untreated rats showed higher levels and impaired glucose tolerance as compared with the normal control rats.

The observed improvement in glycemic response of diabetic rats treated with CAFS supplemented diet was evident by significant increase in serum insulin level and lowering of glucose tolerance curve. Oral glucose tolerance test is a measure of insulin function or the degree of peripheral glucose utilization. The insulin augmenting effect of CAFS in diabetic condition indicates its ability to ameliorate the diabetes induced pancreatic β-cells damage as indicated in the present study. This suggests that CAFS diet may be acting on the pancreas with possible stimulation of either surviving β cells or regenerated β cells of islets of Langerhans to release more insulin [36] and causing significant antihyperglycemic response in diabetic rats. An earlier finding of the presence of heterogenous phytoconstituents with antidiabetic effect in CAFS [19] along with the present findings suggest that CAFS exhibited antidiabetic effect, which causes the reduction of plasma glucose level and lowering of glucose tolerance curve. The potential of CAFS methanolic extract to interfere with the intestinal glucose absorption in the gut via the inhibition of α-amylase and α-glucosidase activities further confirms this view [20]. CAFS supplemented diet lowered blood glucose in STZ-induced diabetic rats effectively and its effect was almost equal to that of standard drug - glibenclamide. Urine glucose study indicated that diabetic rats treated with CAFS supplemented diet produced significant decrease in diabetes induced urine glucose level when compared with the diabetic untreated rats. A marked reduction in blood glucose level and urine glucose level toward normal levels suggested antidiabetic potential of CAFS.

Induction of diabetes by STZ causes loss of body weight due to the increased muscle wasting and loss of tissue proteins [37]. The decrease in body weight and increase in food as well as water intake were commonly observed in diabetes because of β-cells destruction from metabolic changes caused by lack or deficiency of insulin [38]. In the present study, the reduction in body weights of diabetic untreated rats are in accordance with the findings of Pepato *et al*. [39], Nair *et al*. [40], Antai *et al*. [41], Aml *et al*. [42] and Aja *et al*. [43] who attributed the loss in body weight of diabetic animals to the increased degradation of structural proteins during inadequacy of energy metabolism from carbohydrate. Treatment with CAFS supplemented diet however restored the loss in body weight of diabetic rats and produced blood glucose stabilization effect.

Glycogen is a storage form of glucose residues in the liver and is synthesized by glycogen synthase. The quantity of glycogen in various tissues is a direct manifestation of insulin activity, as insulin supports intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase [44]. Thus, liver glycogen level may be considered as the best marker for assessing anti-hyperglycemic activity of any drug [45]. In the present study, the decrease in hepatic glycogen of diabetic untreated rats is in accordance with the findings of Ahmed *et al*. [46] and Nahla *et al*. [47] and supports the suggestion of increased glucose output during insulin deficiency. Several investigators have attributed hepatic glycogen depletion to the loss of glycogen synthase activating system in STZ- diabetic animals [48] and/or increased activity of glycogen phosphorylase and glucose -6-phosphatase in diabetic rats [49, 46]. This decrease could be ascribed to the increase in glycogen breakdown and glucose-6-phosphatase activity as well as decrease in the activity of glucokinase. The present investigation also showed that CAFS supplemented diet induces an increase in liver glycogen content of STZ- diabetic rats. This effect is in accordance with the findings of Nahla *et al*. [47], Lalhlenmawia *et al*. [50] and Waisundara *et al.* [51] who attributed the increase in liver glycogen of diabetic treated with different plants extracts to the increased insulin response, which in turn promotes conversion of inactive form of glycogen synthetase to the active form and enhances conversion of blood glucose into glycogen.

Similar to observations in other reports by Merzouk *et al*. [52], Habibuddin *et al*. [53], Lee *et al*. [54], Malatiali *et al*. [55] and Ren *et al*. [56], the present study showed enlarged liver/body weight and kidney/body weight in diabetic untreated rats. Liver enlargements in diabetic

condition have been attributed to the triglycerides accumulation [52, 54]. In addition, kidney enlargements were ascribed to over-expression of transforming growth factor (TGF) beta-1 in the proximal convoluted tubule cells and glomerular mesengial cells [57] as well as increased Activin beta A in the tubular epithelial cells [56]. On the contrary, the observed marked reduction of pancreas weight/body weight in diabetic untreated rats could be due to distruption and disappearance of pancreatic islets of langerhan [58, 59]. After 28 days of treatment, diabetic rats on glibenclamide/ CAFS diet however reversed the condition. However, the non-significant changes observed in the heart weight/body weight of diabetic untreated rats compared with both diabetic treated (CAFS and glibenclamide) and non-diabetic (normal control) showed that STZ-induction did not have direct effect on the heart weight/ body weight for the period of study. The restoration of pancreas, liver and kidney relative weights of diabetic rats treated with CAFS diet is an indication that CAFS could play vital roles in the improvement of the necrotic of islet of langerhans, hypertrophy of the liver and kidney in diabetic rats.

Diabetes affects both glucose and lipid metabolism [60]. In normal rats, insulin activates the lipolytic hormones action on the peripheral fat, which hydrolyses triglycerides and prevents mobilization of free fatty acids [61]. In diabetes state, insulin deficiency may cause a variety of derangements in metabolic and regulatory processes that leads to accumulation of LDL, total cholesterol and triglycerides levels as well as depleting HDL level [62]. In the present study, total cholesterol, triglycerides, VLDL, and LDL levels increased significantly in liver and plasma of STZ-diabetic rats as compared with the normal ones. These results are in accordance with the finding of Rawi [63] and Lalhlenmawia *et al*. [50] who recorded a marked increase of total lipids in serum and liver of diabetic rats. Several studies have demonstrated that insulin deficiency in STZ -diabetic animals enhanced the breakdown of fat [64, 65], increased the mobilization of free fatty acids from the peripheral depots [66, 67] and caused uninhibited actions of lipolytic hormones (glucagon and catecholamines) on the fat depots [68]. Akah *et al*. [3] linked high levels of triglycerides, LDL-c and VLDL-c with the incidence of heart disease, insulin resistance and diabetes mellitus. Treatment of diabetic rats with CAFS supplemented diet in the present study, significantly inhibited the increase in liver and plasma TC, TG, LDL-c, VLDL-c, atherogenic risk (LDL-c/HDL-c) and coronary risk (TC/HDL-c) indices while reversing the decreased HDL-c in liver and plasma caused by STZ-induction after four weeks of treatment. This lipid lowering action in diabetic condition could be ascribed to the free radicals scavenging

and antioxidant properties of CAFS [20] possibly through the inactivation of hepatic HMG-CoA reductase, a key enzyme, in cholesterol synthesis [69].

The assessment of haematological parameters could be used to reveal the deleterious effect of diabetes conditions in rats. Glycosylated heamoglobin (HbA1C) increased due to the persistent hyperglycemia, which leads to glycation of haemoglobin and hemolysis of red blood cell membrane [70, 71, 27]. In the present study, increase in the level of HbA1C in diabetic untreated rats is an indication of increased reactive oxygen species generated through hyperglycemia, which enhanced the oxidative reactions associated with protein glycation [72, 27] and reduced haemoglobin synthesis [73]. The concentration of HbA1C is related to diabetic retinopathy, nephropathy and neuropathy and it is considered a tool for the diagnosis and prognosis of diabetes-associated complications [74]. Further, the observed reduction of red blood cell and its indices levels in diabetic untreated rats are in accordance with the findings of Stookey *et al.* [75], Muhammad, and Oloyede [76]. Stookey *et al.* [75] ascribed this effect to abnormal hemoglobin synthesis, failure of blood osmoregulation and plasma osmolarity while Muhammad and Oloyede [76] linked this effect to destruction of matured red blood cells, which leads to low Hb and PCV levels. According to Shevchenko and Elfimov [77], decreased Hb, PCV, MCH and MCHC levels in glomectomized diabetic rats are due to suppression of haemopoiesis.

Treatment with CAFS supplemented diet in the current study significantly inhibited the increase in HbA1C and decrease in Hb levels in diabetic rats. The ability of CAFS to decrease HbA1C level in diabetic rats is an indication of its potential to prevent the diabetic-associated complications [74]. These results are in accordance with the findings of Akah *et al.* [3] as well as Ikewuchi and Ikewuchi [78], who reported ameliorative effects on red blood cell and its indices in leaf extract of *Vernonia amygdalina* and rhizomes extract of *Sansevieria senegambica* respectively. This ameliorative effect of CAFS diet could be attributed to its heterogeneous phytoconstituents [19], which could possibly improves bone marrow functions, a major site for erythropoiesis or stimulates the formation / secretion of erythropoietin in the stem cells of the diabetic animals [79]. Erythropoietin is a glycoprotein hormone, which stimulates stem cells in the bone marrow to produce red blood cells [80]. The stimulation of this hormone enhances rapid synthesis of RBC that improved MCH and MCHC levels [81]. These parameters are used to define the concentration of haemoglobin and to suggest the restoration of oxygen carrying capacity of the blood abnormalities in the haematological parameters.

Peripheral white blood count (WBC) and its differentials such as basophils, monocytes, eosinophils, lymphocytes and neutrophils elevation has been shown to be associated with insulin resistance, type 2 diabetes [82] and diabetes micro- and macro-vascular complications [83]. An elevated white blood cell count in peripheral blood is a known risk factor of coronary artery disease [84]. In the present study, the reduced WBC count and its differentials in diabetic rats fed with CAFS supplemented diet is an indication that CAFS could suppresed the diabetic induced elevation of total white blood cell counts. Data obtained in CAFS treated diabetic rats compare favourably with glibenclamide treated rats, confirming its anti-diabetic potency.

Conclusion

In conclusion, CAFS possesses antidiabetic properties possibly due to a combination of mechanisms, which include stimulation of insulin secretion from the existing beta cells of the pancreas, prevention of glycosylation of red blood cell; protection against diabetic induced tissues damage, lipid disorders and alterations of haematological parameters. Thus, CAFS diet could possibly be used to complement the management of diabetes mellitus.

Recommendations

Further studies are required to explore the role of CAFS in the treatment of various diabetes complications other nutritionally related diseases.

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