Effects of *Buchholzia coriacea* Seed on Nutrient Utilization and Serum Biochemical Parameters in Alloxan-Induced Diabetic Rat

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Abstract: The effects of *Buchholzia coriacea* seed on the nutrient utilization and biochemical parameters in alloxan-induced diabetic rat were evaluated. *Buchholzia coriacea* (BC) seed was washed, sliced, dried, pulverized and mixed with standard ration at 2.5%, 5%, 10% and 20%. The proximate nutrient composition of the standard and prepared rations was determined. The *Buchholzia coriacea* incorporated rations and standard ration were fed to alloxan-induced diabetic rats for 70 consecutive days. The nutrient utilization and biochemical parameters as well as the histopathology of pancreas of the treated rats were evaluated. The *Buchholzia coriacea* at 2.5% inclusion rate significantly ($p < 0.05$) improved the nutrient utilization and biochemical parameters that were compromised in diabetic rats fed with standard ration alone. The *B. coriacea* also reversed the pancreatic islet damage induced by alloxan. *Buchholzia coriacea* have potent antidiabetic and hypolipidemic activities and should not be incorporation in excess of 5% in the diet.

Keywords: Apparent digestibility, biological value, hypolipidemia, gas chromatography, glycosylated hemoglobin.

INTRODUCTION

Buchholzia coriacea Engl (wonderful kola) is a member of the family *Capparidaceae* and is found in Guinea, Ghana, Liberia, Cameroon, Gabon and Southern Part of Nigeria [1]. It is an evergreen tree (10 - 20 metre high) with smooth, blackish-brown stem, large glossy pinnate leaf and conspicuous white or yellow flower at the terminal of the branches. The seed has sharp pungent taste and traditionally used as a spice and medicine [2]. The seed is used as aphrodisiac in Cameroon, childbirth enhancer in Ivory Coast and antidiabetic agent in Nigerian ethnomedical practice. The antiulcer, analgesic, antidiabetic, anthelminthic, antibacterial, anti-inflammaory and antioxidant activities of *B. coriacea* have been reported [3-9]. There is paucity of information on the effects of *Buchholzia coriacea* on nutrient utilization and clinical biochemical parameters in alloxan-induced diabetes mellitus. This study was designed to investigate the effects of *Buchholzia coriacea* seed on nutrient utilization and clinical biochemical parameters in alloxan-induced diabetic rat.

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MATERIALS AND METHODS

Plant Collection and Extract Preparation

Fresh *Buchholzia coriacea* seeds were procured from Abakpa Market Abakaliki, Ebonyi state, Nigeria and authenticated by a botanist. It was peeled, washed, and cut into smaller pieces before air drying under a shade for 10 days at environmental temperature (25 $\pm$ 2 ^{0}C). The seeds were pulverized and stored in air tight container before usage. The pulverized seed was extracted with soxhlet apparatus maintained at 40 ^{0}C using acetone for 24 h. The extract was concentrated with rotary evaporator at 40 ^{0}C.

Gas Chromatography/Mass Spectrometry Analysis

The bioactive compounds in acetone extracts of *Buchholzia coriacea* seed was analyzed using gas chromatography/mass spectrometry (QP 2010 Plus Shimadzu, Japan) equipped with a flame ionization detector (FID). Helium was used as carrier gas and at a flow rate of 3.0 mL/min. The column temperature was programmed from 70 to 280°C at the rate of 5°C/min. Injector and detector temperatures were set at 250 and 260°C, respectively. All quantifications were carried out using a built-in data-handling program provided by the manufacturer of the GC (Perkin Elmer, Norwalk, CT, USA). Interpretation of mass spectrum from gas chromatography-mass spectrum was conducted using

the database National Institute Standard and Technology (NIST) version 2.0, 2009 library.

Formulation of Experimental Diet

The diets were formulated as described by Amaechi *et al*. [10]. Briefly, standard diet containing 83% corn starch, 10% casein, 2% vegetable oil, 3% rice bran and 2% vitamin and mineral mix was prepared. The dried pulverized *Buchholzia coriacea* (BC) seed was incorporated at different concentrations of 2.5%, 50%, 10% and 20% to standard ration.

Experimental Animals

Weanling albino Wistar rats (22-26 days old) of both sexes with average initial body weight of 40 $\pm$ 2 gram were used for the experiments. The rats were obtained from a reputable source and housed in stainless steel metabolism cages maintained at an ambient temperature of 28 $\pm$ 2 $^{\circ}$C and natural light-dark cycle. All the experimental animals were acclimatized for 14 days on the standard diet. The rats were maintained in accordance with the recommendation in the guide for the care and use of laboratory animals [11]. The experimental protocol was approved by the Institutional Ethics Committee.

Proximate Analysis of Diet Samples

Proximate composition of the various diet samples were analyzed by the methods described by James [12].

Induction of Diabetes

Diabetes was induced by intraperitoneal injection of alloxan (160 mg/kg) to overnight (16 h) fasted rats with free access to water. After 72 h, fasting blood glucose levels of the rats were determined with a glucometer (Accu-check active, Germany).

Experimental Design

The method described by Pellet and Young [13] was adopted. Eight (8) normoglycemic and 40 diabetic rats were used for the experiment. The normoglycemic rats were assigned group I and diabetic rats were randomly assigned to 5 groups (II - VI) of 8 rats each. The rats were individually housed in metabolic cage. Groups I and II were fed with standard feed while groups II – VI were fed with feed containing 2.5, 5, 10 and 20% BC seed, respectively. The rats were fed *ad libitum* for 70 consecutive days. The daily feed intake was recorded, while the body weight was recorded at weekly interval. The daily fecal output was recorded, collected and air dried while the daily urine output was recorded, pooled and stored by addition of 1 ml of 0.1 N HCl in brown bottle nitrogen analysis. After the 70th day of feeding, the rats were fasted for 16 h and blood samples were collected through ocular puncture into EDTA and plain bottle for whole blood and serum preparation respectively. Thereafter, the rats were sacrificed by cervical dislocation, immediately laparotomised and pancreas were excised and preserved in 10% formalin solution for histopathological examination.

Evaluation of Nutrient utilization

Nitrogen content of both urine and feacal samples was analyzed using the kjeldahl method as described by James [12]. Protein efficiency ratio (PER), feed efficiency ratio (FER), apparent digestibility (AD), true digestibility (TD), biological value (BV) and net protein utilization (NPU) were calculated.

Serum Biochemical Analysis

Fasting blood glucose (FBG) was determined using a glucometer (Accu- check, Germany). Glycosylated hemoglobin (HbA1c) was analyzed by the method of Trivelli *et al.* [14], using a glycohemoglobin test kit (Teco Diagnostics, USA). The serum total cholesterol (TC), triglycerides (TG) high density lipoprotein cholesterol (HDL-C) were analysed using a commercial available diagnostic test kit (Randox Laboratory, UK). Serum low density lipoprotein cholesterol (LDL-C) was calculated using Fridewald equation [15]:

LDL-C (mg/dl) = Total cholesterol – (Triglycerides/5) - HDL-C.

Histopathology

The Pancreas of the rats were excised and fixed in 10% formol saline for 24 h. They were washed in ascending grades of ethanol, cleared with xylene, embedded in paraffin wax, sectioned with a microtome, and stained with hematoxylin and eosin (H and E) and mounted on Canada balsam (Sigma-Aldrich, St. Louis, MO) [16]. All the sections were examined under a light microscope at ×400 magnification. Photomicrographs of lesions were taken with an Olympus photo microscope (Olympus Scientific Equipment, Ashburn, VA) for observations of the histopathological lesions.

Statistical Analysis

One way analysis of variance (ANOVA) followed by Duncan's Post hoc test was used to separate the

means. Data obtained were expressed as mean ± standard deviation and differences in means were considered to be significant at $p < 0.05$. The statistical software used was SPSS version 20.

RESULT

Bioactive Compounds Identified in the Acetone Extract of *Buchholzia coriacea* Seeds

The GC/MS analysis of the acetone extract of *B. coriacea* showed the presence of nine compounds. Their retention time, peak area, molecular weight and formulae are presented in Table **1**. 9-Octadecanoic acid (Z) (Oleic acid) and 9,12-Octadecadienoyl chloride (Z,Z) (Linoleic acid chloride) were the most abundant with the peak areas of 39.22% and 39.35% respectively.

Proximate Composition of Standard Ration and Rations Containing *B. coriacea* at Varied Concentrations

The incorporation of *B. coriacea* caused concentration-dependent ($p < 0.05$) increase in the proximate moisture, fat, ash, crude fiber and protein content of the treated rations when compared with the standard ration. The carbohydrate content of the *B. coriacea*-treated rations decreased ($p < 0.05$) in concentration dependent manner when compared with the standard ration (Table **2**).

Effect of *B. coriacea* on the Nutrient Utilization in Diabetic Rats

The body mass gain (BMG), protein efficiency ratio (PER), feed efficiency ratio (FER), urinary nitrogen (UN), endogenous urinary nitrogen (EUF), endogenous fecal nitrogen (EFN), percentage nitrogen retention

Table 1: Bioactive Compounds Identified in the Acetone Extract of *Buchholzia coriacea* Seeds

Peak No	Retention Time (Minutes)	Identity of Compound	Peak Area (%)	Molecular Weight	Molecular Formular
1	3.64	Trimethylsilylmethanol	1.58	104	$C_4H_{12}OSi$
2	8.63	2,6-octadien-1-ol-3,7-dimethyl (E) trans geraniol (Lemonol or Nerol)	0.40	154	$C_{10}H_{18}O$
3	8.88	2,6-Octadienal-3,7-dimethyl-(E) α citral	0.89	152	$C_{10}H_{16}O_2$
4	14.05	α-(4methyl-3-pentenyl) oxirane methanol	1.58	170	$C_{10}H_{18}O_2$
5	20.90	9-Octadecenoic acid, methyl ester (Oleic acid methyl ester)	1.14	296	$C_{19}H_{36}O_2$
6	22.12	9-Octadecanoic acid (Z) (Oleic acid)	39.22	282	$C_{18}H_{34}O_2$
7	23.32	Hexadecanoic acid, 2-hydroxy-1, 3-propanediyl ester	8.86	568	$C_{35}H_{68}O_5$
8	24.88	9,12-Octadecadienoyl chloride (Z,Z) (Linoleic acid chloride)	39.35	298	$C_{18}H_{31}ClO$
9	25.03	Octadecanoic acid, 2-hydroxy-1, 3-propanediyl ester	7.60	624	$C_{39}H_{76}O_5$

Table 2: Proximate Composition of Standard Ration and Rations Containing *B. coriacea* at Varied Concentrations

Parameter	SD	2.5% BC	5% BC	10% BC	20% BC
Moisture	6.52 ± 0.18^e	7.79 ± 0.12^d	9.76 ± 0.09^c	11.21 ± 0.21^b	13.23 ± 0.22^a
Ash	2.58 ± 0.13^d	2.83 ± 0.13^c	2.84 ± 0.08^c	2.98 ± 0.13^b	3.31 ± 0.17^a
Crude fiber	2.60 ± 0.07^d	2.87 ± 0.06^c	3.49 ± 0.08^b	4.16 ± 0.19^a	4.26 ± 0.29^a
fat	2.68 ± 0.04^e	3.63 ± 0.22^d	4.23 ± 0.09^c	4.88 ± 0.22^b	5.81 ± 0.28^a
Crude protein	16.95 ± 0.05^e	19.21 ± 0.22^d	21.23 ± 0.25^c	23.45 ± 0.65^b	25.17 ± 0.17^a
CHO	68.67 ± 0.28^a	63.67 ± 0.22^b	58.44 ± 0.31^c	51.87 ± 2.58^d	48.24 ± 0.66^e

Values are means of triplicate determinations ± standard deviation. Means in the same row bearing different superscripts are significantly different ($p < 0.05$). SD = standard diet, BC = *Buchholzia coriacea,* CHO = carbohydrate.

(NR%), apparent digestibility (AD) and true digestibility (TD) of the diabetic control group were lower ($p< 0.05$) compared with the positive control group while the feed intake (FI), fecal nitrogen (FN), nitrogen intake (NI), nitrogen retention (NR), net protein utilization (NPU) and biological value (BV) of the diabetic control group were higher ($p < 0.05$) compared with the positive control group. The protein intake (PI), FI, BMG. NI, NR, EUN and EFN of 2.5% and 5% BC treated groups were higher ($p < 0.05$) when compared with the positive and diabetic control group. The FI, PI, BMG, NI, and NR of the 10% and 20% BC treated groups were lower ($p < 0.05$) when compared with the diabetic control group. The FN and UN of the BC treated groups were higher ($p < 0.05$) when compared with the diabetic and positive control groups. The 5%, 10% and 20% BC caused significant ($p < 0.05$) concentration-dependent decrease in the AD, TD, NPU and NR% of the treated groups when compared with the diabetic and positive control groups (Table **3**).

Effects of *B. coriacea* on Biochemical Parameters in Diabetic Rats

The HbA1c, FBG, TC, TG and LDL-C of the diabetic control group were higher ($p < 0.05$) when compared with the positive control group, while the HDL-C of the diabetic control group was lower ($p < 0.05$) compared with the positive control. The BC caused significant ($p < 0.05$) decrease in the HbA1c, FBG, TC, TG and LDL-C in a concentration-dependent manner when compared with the diabetic control group. The HDL-C of BC treated groups were higher ($p < 0.05$) in a concentration-dependent manner when compared with the diabetic control group. The HbA1c, FBG, TC, TG, HDL-C and LDL-C of the 20% BC treated group were comparable to the positive control group (Table **4**).

Histopathology

The diabetic control group showed no observable pancreatic islet while the *B. coriacea* treatment caused concentration-dependent increase in the pancreatic islet cells area and density which were comparable to the positive control group (Figure **1**).

DISCUSSION

The acetone *B. coriacea* seed elicited hypolipidemic and antidiabetic activities as well as reversed pancreatic damage and improved nutrient utilization in alloxan-induced diabetic rats. The hypolipidemic and antidiabetic activities of *B. coriacea* are suggested to be mediated by oleic acid and linoleic acid derivatives.

Table 3: Effect of *B. coriacea* on the Nutrient Utilization in Diabetic Rats

parameters	Normal control	Diabetic control	2.5% BC	5% BC	10% BC	20% BC
FI (g)	282.38 ± 38.77^{d}	330.05 ± 25.85^{b}	372.50 ± 11.02^{a}	297.93 ± 10.74^{cd}	233.50 ± 7.42^{e}	188.32 ± 13.04^{f}
PI (g)	47.84 ± 6.57^{d}	56.02 ± 4.38^{c}	72.18 ± 2.24^{a}	63.97 ± 2.31^{b}	54.04 ± 1.72^{c}	47.66 ± 3.30^{d}
BMG (g)	87.57 ± 10.40^{bc}	80.23 ± 2.79^{c}	105.60 ± 5.20^{a}	88.22 ± 6.81^{b}	57.72 ± 4.52^{d}	44.13 ± 6.82^{e}
PER	1.85 ± 0.07^{a}	1.44 ± 0.09^{b}	1.46 ± 0.03^{b}	1.38 ± 0.06^{b}	1.08 ± 0.08^{c}	0.92 ± 0.09^{d}
FER	0.31 ± 0.01^{ab}	0.24 ± 0.02^{c}	0.28 ± 0.01^{b}	0.30 ± 0.01^{b}	0.25 ± 0.02^{c}	0.23 ± 0.02^{c}
NI (mg)	1297.76 ± 178.21^{d}	1519.77 ± 118.77^{c}	1958.28 ± 60.84^{a}	1735.36 ± 62.59^{b}	1466.01 ± 46.59^{c}	1293.10 ± 89.52^{d}
FN (mg)	108.22 ± 24.85^{f}	155.64 ± 16.48^{e}	174.15 ± 13.03^{d}	218.02 ± 13.19^{c}	234.21 ± 8.85^{b}	264.28 ± 13.81^{a}
UN (mg)	41.10 ± 3.51^{e}	38.97 ± 0.05^{e}	69.37 ± 1.99^{a}	61.47 ± 0.73^{b}	51.93 ± 0.32^{c}	45.81 ± 2.81^{d}
EFN (mg)	39.19 ± 4.37^{c}	35.99 ± 1.29^{d}	51.48 ± 2.32^{a}	45.29 ± 2.52^{b}	34.90 ± 2.02^{de}	30.69 ± 3.05^{e}
EUN (mg)	11.93 ± 1.75^{c}	10.45 ± 0.52^{d}	16.83 ± 0.95^{a}	14.37 ± 1.01^{b}	10.21 ± 0.80^{d}	8.51 ± 1.22^{e}
NR	1148.78 ± 153.22^{d}	1325.16 ± 102.57^{c}	1714.76 ± 48.89^{a}	1455.87 ± 50.21^{b}	1179.86 ± 38.14^{d}	984.01 ± 80.71^{e}
NR%	88.56 ± 0.40^{a}	87.20 ± 0.20^{b}	87.57 ± 0.33^{b}	83.90 ± 0.27^{c}	80.48 ± 0.17^{d}	75.97 ± 1.15^{e}
AD%	91.77 ± 0.76^{a}	89.78 ± 0.32^{c}	91.12 ± 0.41^{b}	87.45 ± 0.35^{d}	84.70 ± 0.18^{e}	79.53 ± 0.98^{f}
TD	88.74 ± 0.65^{a}	87.43 ± 0.28^{b}	88.49 ± 0.43^{a}	84.84 ± 0.39^{c}	81.64 ± 0.23^{d}	77.16 ± 0.97^{e}
BV	95.30 ± 0.46^{bc}	96.31 ± 0.34^{a}	95.03 ± 0.08^{bc}	95.01 ± 0.47^{bc}	94.81 ± 0.14^{bc}	94.56 ± 0.28^{d}
NPU	84.56 ± 0.37^{ab}	$87.50 \pm .15^{a}$	84.09 ± 0.36^{b}	80.61 ± 0.35^{c}	77.40 ± 0.25^{d}	76.28 ± 7.75^{d}

Values are means ± standard deviation. Means in the same row bearing different superscripts are significantly different ($p < 0.05$). BC = *Buchholzia coriacea*, FI = feed intake, PI = protein intake, BMG = body mass gain, PER = protein efficiency ratio, FER = feed efficiency ratio, NI = nitrogen intake, FN = fecal nitrogen, UN = urinary nitrogen, EFN = endogenous fecal nitrogen, EUN = endogenous urinary nitrogen, AD = apparent digestibility, TD = true digestibility, BV = biological value, NPU = net protein utilization, NR = nitrogen retention, %NR = percentage nitrogen retention.

Table 4: Effects of *B. coriacea* on Biochemical Parameters in Diabetic Rats

Parameter	Normal control	Diabetic control	2.5% BC	5% BC	10% BC	20% BC
HbA1c (%)	4.05 ± 0.10^{d}	10.65 ± 1.99^{a}	8.70 ± 0.73^{b}	7.43 ± 1.59^{b}	6.60 ± 0.07^{c}	5.80 ± 0.54^{d}
FBG (mg/dl)	93.17 ± 12.80^{d}	212.50 ± 35.86^{a}	152.83 ± 6.74^{b}	138.67 ± 3.72^{c}	100.83 ± 9.50^{d}	90.50 ± 11.91^{d}
TC (mg/dl)	165.39 ± 14.18^{e}	251.28 ± 13.47^{a}	224.62 ± 9.61^{b}	219.23 ± 8.16^{bc}	203.07 ± 10.44^{d}	168.59 ± 13.63^{e}
TG (mg/dl)	117.82 ± 6.70^{d}	195.77 ± 5.88^{a}	172.08 ± 4.78^{b}	136.15 ± 6.18^{c}	121.54 ± 20.15^{de}	100.70 ± 9.10^{e}
HDL-C (mg/dl)	54.14 ± 6.40b^{c}	27.44 ± 8.27^{d}	34.31 ± 5.26^{d}	47.81 ± 1.70^{c}	61.26 ± 5.53^{b}	69.94 ± 11.74^{a}
LDL-C (mg/dl)	86.44 ± 11.99^{e}	184.69 ± 14.24^{a}	155.89 ± 13.38^{b}	144.19 ± 6.84^{c}	126.85 ± 13.96^{d}	78.51 ± 20.38^{e}

Values are means ± standard deviation. Means in the same row bearing different superscripts are significantly different (p< 0.05). BC = *Buchholzia coriacea*, HbA1c = glycosylated hemoglobin, FBG = fasting blood glucose, TC = total cholesterol, TG = triglycerides, HDL = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol.

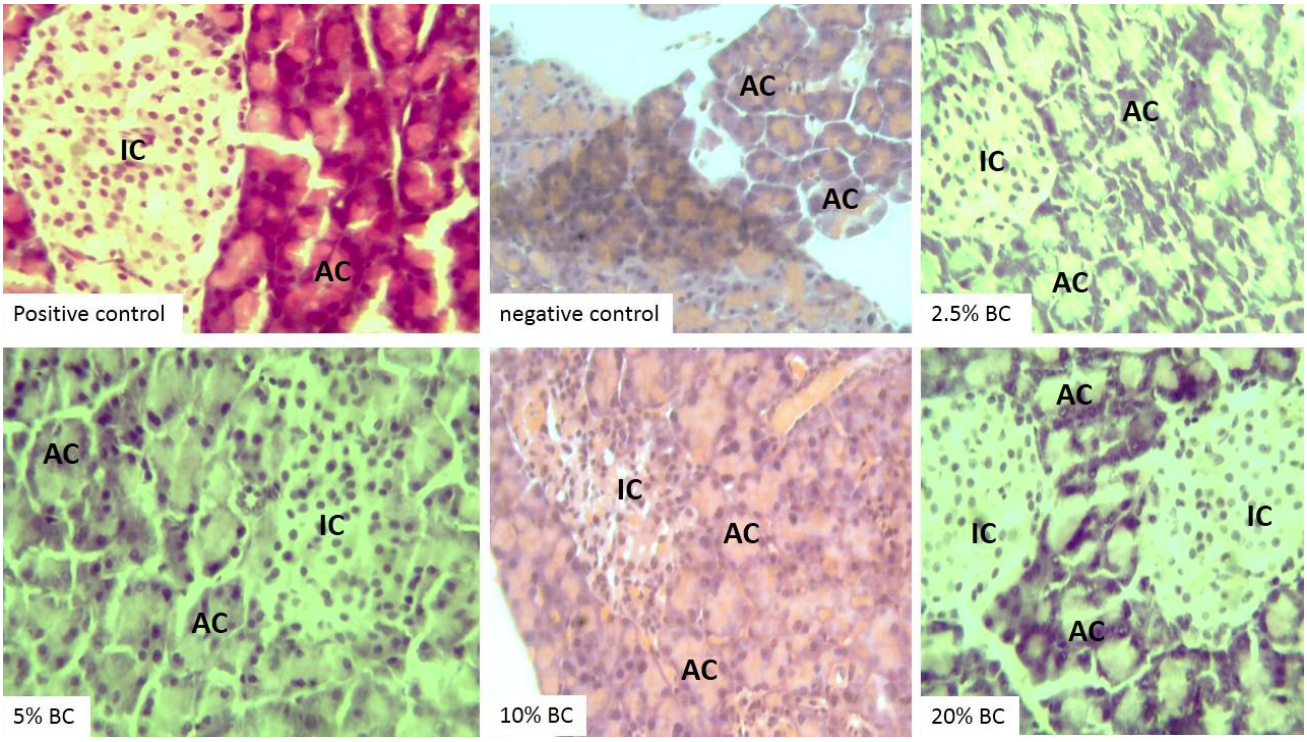

Figure 1: Photomicrograph sections (H and E ×400) of pancreas of normal and diabetic rats.

Legend: IC = islet cell, AC = acini cell.

The GC-MS analysis showed the presence of mainly Oleic acid, hexadecanoic acids and the derivatives which were in agreement with the report of Duru *et al*. [17] on the chemical composition of *Buchholzia coriacea* seed. These compounds have been reported to possess anti-inflammatory, antioxidant and hypocholesterolemic activities [18]. Anti-inflammatory, antioxidant and hypocholesterolemic agents have been shown to promote healthy living in diabetic patients [19].

The supplementation of the ration with BC increased the ash, crude fibre, fat, and crude protein content in a concentration dependent, which corroborates the report of Nnamani [20]. He noted that vegetables are cheap source of vitamins, mineral and amino acids. The increased crude fibre content may act as bulk laxative; which will reduce intestinal transit time, nutrient digestion and absorption [21].

The higher feed intake in the diabetic control is attributed to poor glucose uptake by cell as a result of insulin resistance or deficiency which characterize diabetes mellitus [22]. The decreased feed intake at 10% and 20% BC supplementation could be attributed to high fiber content which leads to satiation and satiety [23, 24] and/or poor palatability of the feed. The decrease in the BMG, FER and PER (Table **4**) of the diabetic control group is due to muscle wasting which characterize alloxan induced diabetic condition [25].

The increase in BMG, FER and PER observed in 2.5% VA groups treated group may be attributed to the antidiabetic potential of BC [9].

The BC supplementation in the ration reduced the FBG, Hba1c, TC, TG, LDL-C and increase in HDL-C level in the treated rats when compared to the diabetic control group (Table **5**). This could be attributed to increase in insulin secretion and/or sensitivity [26]. The BC caused concentration-dependent regeneration of the pancreatic islet cells damaged by alloxan administration (Figure **1**). Pancreatic islet cell regeneration is associated with increased insulin secretion, enhanced glucose uptake, decreased serum glucose level and reduced glycosylation of hemoglobin [27]. The pancreatic islet cell regeneration may be linked to the antioxidant activity of BC [28]. The antioxidant retard the spontaneous production of free radical and cell membrane damage induced by alloxan in the treated rats [29].

The BC produced hypocholesterolaemic and hypotriglyceridemic effects in the alloxan-induced diabetic rats. Hypercholesterolemia and hypertriglyceridemia is the major risk factor for cardiovascular diseases; common complication of diabetes mellitus [30, 31]. This indicates that BC could be employed in the treatment and/or prevention of complications of diabetes mellitus. The mechanism of the hypolipidemic effects of BC has not been elucidated but could be linked to enhanced insulin production and activation of lipoprotein lipase [32]. The hypolipidemic effects of BC supplemented ration corroborates with high dietary fiber content of the ration. Dietary fiber lower feed intake, reduce cholesterol and triglyceride absorption and elevate faecal bile acid and cholesterol excretion [33]. The hypolipidemic effects of B. coriacea is in agreement with the report of Olaiya and Omolekan [34] on the antihypercholesterolemic activity of ethanolic extract of *Buchholzia coriacea* in rats.

CONCLUSION

The extracts produced hypolipidemic and antidiabetic activities as well as reversed pancreatic islet cell damage in alloxan-induced diabetic rats. This study justifies the use of *Buchholzia coriacea* in the folkloric management of diabetic mellitus and suggests that its incorporation in excess of 5% in the diet should be avoided.

DECLARATION OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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