

Antidiabetic activities of chloroform fraction of *Anthocleista vogelii* Planch root bark in rats with diet- and alloxan-induced obesity-diabetes

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Abstract

Ethnopharmacological relevance: *Anthocleista vogelii* Planch is a medicinal plant traditionally used in West Africa for the management and treatment of diabetes mellitus.

Aim of the study: To determine the antidiabetic activities of chloroform fraction (CF) of *Anthocleista vogelii* Planch root bark in rats with diet- and alloxan-induced obesity-diabetes.

Materials and Methods: Inhibitory activities of CF against α -amylase and α -glucosidase activities were determined *in vitro*. Three weeks old rats were fed with high-fat diet for 9 weeks to induce obesity prior to further induction of diabetes using alloxan (150 mg/kg body weight, i.p.). Blood glucose levels and body weight were measured every 7 days throughout the experiment. Glucose tolerance was assessed in normal and CF-treated rats on day 21. Terminal blood samples were collected from sacrificed animals for the measurement of serum insulin levels. Pancreases were excised from treated and untreated animals for histopathological examination.

Results: LCMS/MS chromatographic profile of CF via positive and negative modes revealed 13 and 23 compounds respectively. Further analysis revealed quebrachitol (QCT), loganin, sweroside, oleoside 11-methyl ester and ferulic acid, which have been previously reported for their antidiabetic activities, as constituents of CF. CF inhibited activities of α -amylase ($IC_{50} = 51.60 \pm 0.92 \mu\text{g/ml}$) and α -glucosidase ($IC_{50} = 5.86 \pm 0.97 \mu\text{g/ml}$) in a dose-dependent manner. Treatment of animals with obesity-diabetes with 100 and 200 mg/kg CF significantly improved glucose tolerance ($P < 0.001$) and enhanced serum insulin levels ($P < 0.05$) compared to diabetic control rats.

Conclusions: Antidiabetic activities of CF might be mediated via inhibition of α -amylase and α -glucosidase activities, and enhancement of insulin and leptin sensitivity in obesity-diabetes rats. This study further substantiates the traditional use of *A. vogelii* in the management and treatment of diabetes in Africa and encourages further studies to investigate its mechanism of action.

Key words: Antidiabetic; *Anthocleista vogelii*; amylase; alloxan; glucosidase; LCMS

Chemical compounds studied in this article:

(+)-Maackiain 3-O-glucoside (PubChem CID: 44257441); 3-bromo-2E-acrylic acid (not found); 3-Hydroxy-3-methyl-glutaric acid (PubChem CID: 1662); 5,7-Dihydroxy-3',4'-dimethoxy-6,8-dimethylflavone (not found); 5,7-Dihydroxy-3-methoxy-4'-prenyloxyflavone (not found); 5-Acetylamino-6-formylamino-3-methyluracil (PubChem CID: 108214); alpha-Bromocrotonic acid (PubChem CID: 5312952); Apigenin 7-methyl ether 5-glucoside (not found); C16 Sphinganine (PubChem CID: 70679052); D-Glucoside (PubChem CID: 64947); Di-alpha-furfuryl ether (PubChem CID: 263034); Emmotin A (PubChem CID: 42608142); Ferulic acid (PubChem CID: 445858); Galactopinitol B (PubChem CID: 101926786); gamma-Thujaplicin (PubChem CID: 12649); Loganin (PubChem CID: 87691); Methylorsellinic Acid, Ethyl Ester (PubChem CID: 3084545); N-hexanoyl-L-Homoserine lactone (PubChem CID: 10058590); Oleamide (PubChem CID: 5283387); Oleamide (PubChem CID: 5283387); Oleoside 11-methyl ester (PubChem CID: 10692563); Phellodensin D (PubChem CID: 5316782); Phlorisobutyrophenone (PubChem CID: 5326317); Phytosphingosine (PubChem CID: 122121); Quebrachitol (PubChem CID: 151108); Samidin (PubChem CID: 442150); Sweroside (PubChem CID: 161036); Theobromine (PubChem CID: 5429); Undecanedioic acid (PubChem CID: 15816).

1. Introduction

Diabetes mellitus refers to a metabolic disorder arising from defects in insulin secretion, insulin action or both, resulting in multiple etiology characterized by chronic hyperglycemia and disturbances of fat, carbohydrate and protein metabolism (Alberti and Zimmet, 1998). Diabetes complications includes heart failure, cardiovascular diseases, adverse drug reactions, infections, and early mortality (Thomas et al., 2015).

Several studies have reported increase in the prevalence of type 2 diabetes in many countries. According to Jansson et al. (2015), 382 million people worldwide suffer from diabetes mellitus in 2014 and this number has been projected to reach 600 million people by 2035 (Guariguata et al., 2014). According to WHO (2016), the global incidence of the disease in adults also increased from 108 million in 1980 to 422 million in 2014. Currently, about 425 million people suffer from diabetes globally and 3.1% of this number, representing about 15.9 million people, live in Africa (IDF, 2017). As the incidence of the disease increases, so are the associated costs of managing the disease, with more impact felt in developing countries where resources are scarce (Thomas et al., 2015). Global diabetes-related death was reported to increase from 1.5 million in 2012 to 1.6 million in 2015, with about 2.2 million deaths attributed hyperglycemia in 2012 (WHO, 2016).

Currently available drugs for treating type 2 diabetes include α -glucosidase inhibitors, dipeptidyl peptidase-4 (DPP-4) inhibitors, biguanides, sodium glucose transporter 2 inhibitors and glucagon-like peptide-1 (GLP-1) receptor agonists. However, challenges such as obvious side effects, cost, unavailability in rural areas where they are mostly needed and inability to restore

normoglycemia detract hugely from the therapeutic utility and efficacy of these drugs. This also motivates interests in developing cheaper, more effective and safer antidiabetic agents.

Documented evidence for the use of plant extracts or phytochemicals purified from plant-based materials is increasing. The greatest success in this regard is the development and current clinical use of metformin, isolated from extracts of *Galega officinallis*, for the treatment of type 2 diabetes (Bailey and Day, 2004). *Anthocleista vogelii* Planch, of the Gentianaceae family, is a medicinal plant commonly used in West Africa for treating constipation, malaria fever, typhoid fever, hypertension and diabetes (Kadiri 2009; Musa *et al.* 2010; Olubomehin *et al.*, 2013). Jiofack *et al.* (2010) reported the use of *A. vogelii* stem bark and leaves as a decoction for the treatment of diabetes in traditional medicine practice. The aqueous extract of *A. vogelii* roots has been shown to possess hypoglycemic activity both in normal and hyperglycemic mice, rats and rabbits (Abuh *et al.*, 1990). In a different study, extracts of the leaves, stem bark and whole root of the plant *A. vogelii* have been shown to modulate α -amylase activities (Olubomehin *et al.*, 2013) while the methanolic extract of *A. vogelii* stem bark has been reported to exhibit antidiabetic effects in alloxan-induced diabetic rats (Osadebe, 2014). However, mechanisms underlying actions of extract of *A. vogelii* are poorly understood. This study focused on the characterization of active constituents of *A. vogelii* root bark extract and examined its effects on activities of enzymes involved in starch digestion *in vitro*. Effects of the plant extract on glucose tolerance in animals with type 2 diabetes were also assessed.

2. Methodology

2.1 Plant material

Fresh root bark of *A. vogelii* was harvested in June 2015 from Umuekwune, Ngor-Okpala, Imo State, Nigeria. The plant was authenticated by Dr J. O. Ihuma of the Department of Biological Sciences, Bingham University, Karu, Nigeria (voucher number GA134-7421) and specimens were deposited in Department of Biological Sciences of the University.

2.2 Preparation of chloroform fraction

Pulverized dried root bark of *A. vogelii* (5 kg) was macerated in methanol for 3 days and the filtrate was concentrated using rotary evaporator. The methanolic extract (26.5%) was defatted by macerating in n-hexane and stirring vigorously at intervals for 24 hrs. The process was repeated several times and the n-hexane solution was filtered. The methanolic marc was suspended in water and subsequent extraction with chloroform (3x500 ml) by solvent partitioning using separating funnel. The chloroform fraction (CF) was concentrated using rotary evaporator at 40°C to give a percentage yield of 6.7% stored at -20°C. A pilot study was conducted to investigate effects of the methanolic extract, the n-hexane fraction, chloroform fraction and the aqueous fraction on the inhibition of α -amylase and α -glucosidase. Based on the results of this preliminary studies (Table S1), CF was selected for further studies.

2.3 Acute oral toxicity evaluation

The chloroform fraction of *A. vogelii* was evaluated for acute oral toxicity in conformity with OECD (2002) guidelines. Male Sprague–Dawley rats (200 \pm 10 g) were randomly selected, marked and kept in cages of 5 rats per group. After overnight fasting, rats received a single oral administration of chloroform fraction of *A. vogelii* (300 and 2000 mg/kg body weight) using distilled water as vehicle. Rats were observed for 1, 3 and 14 days respectively for behavioral and clinical signs of toxicity.

2.4 LC-MS/MS analysis of CF from *A. vogelii*

The chloroform fraction of *A. vogelii* was diluted (10-fold) with methanol and analyzed using Agilent 1290 Infinity LC system coupled to Agilent 6520 Accurate-Mass Q-TOF mass spectrometer with dual ESI source. The chromatographic separation was carried out using Agilent Zorbax Eclipse XDB-C18 column (Narrow-Bore 2.1x150mm, 3.5 micron). The column temperature was set at 25⁰C while the Autosampler temperature was 23⁰C at flow rate of 0.5ml/min. Separation was carried out using 0.1% formic acid in water (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B). Following sample injection (1µl), both positive and negative polarity were run in the ESI-MS experiment for 25 min run and 5 min post run times. MS analyses were performed using full scan mode of mass range 100 – 3200 m/z, and 119.03632 and 966.000725 reference ions. Agilent MassHunter Qualitative Analysis B.05.00 (Method: Metabolomics-July2015.m) was used to process the data. The analysis of CF was performed in School of Pharmacy, Monash University Malaysia, Selangor, Malaysia.

2.5 Alpha amylase inhibitory assay

Effects of CF on α -amylase activities was investigated using the starch-iodine test as described by Xiao et al, (2006) with slight modifications. The reaction mixture, comprising of 25 µl of 0.02 M sodium phosphate buffer pH 7.5, 20µl of soluble starch (1%, w/v) and 20µl of either CF or acarbose (control), was incubated for 5 min at 37°C in a 96-well microtitre plate. Amylase solution (6mg/ml, 15 µl) was added to the reaction mixture and incubated for 15 min at 37°C.

This was followed by the addition of 1 M HCl (20µl) to stop the enzymatic reaction. The absorbance reading was measured at 620 nm using Synergy HT BioTek[®] USA microtitre plate reader after the addition of 100µl of iodine reagent. The absorbance reading for test samples was expressed as a percentage of the absorbance of incubations in the presence of acarbose.

2.6 Effects of CF on alpha-glucosidase activities

α-glucosidase activities were measured as described by Johnson *et al.* (2013). The reaction mixture included CF or acarbose (50 µl), 50µl buffer solution (0.1M sodium phosphate buffer, pH 6.9) and 100 µl of a 1.0 U/ml α-glucosidase solution in 96 well plate was incubated at 25 °C for 10min. Then, 5 mM p-nitrophenyl-α-D-glucopyranoside solution (50 µl) was added to the reaction mixture and incubated at 25°C for 5 min. The absorbance was measured at 405 nm before initial and after final incubation using Synergy HT BioTek[®] USA microtitre plate reader. The difference between the change in absorbance readings of the sample and control was expressed as a percentage of the change in absorbance for control incubations.

2.7 Induction of obesity-diabetes

Fifty male Sprague–Dawley rats (30 ± 10 g) were selected from a batch bred within the Animal House, Department of Pharmacy, COMSATS Institute of Information Technology, Abbottabad, Pakistan. Animals were maintained with access to food and water *ad libitum* under controlled temperature (23 ± 2°C) and lighting (12:12 h light/dark cycle). A group of selected rats were fed high-fat diet containing 45% kcal fat, 35% kcal carbohydrate and 20% kcal protein for 9 weeks prior to experimentation to induce obesity (Ojo et al., 2015). Experimental procedures followed the European Community guidelines (EEC Directive of 1986; 86/609/EEC) for animal handling and ethical approval number (PHM-0024/EC/M-4-5.15) was given by the Research Ethics

Committee of Pharmacy Department, CIIT, Abbottabad. Following a 12 h fast, high-fat fed rats included in the study also received a single i.p. injection of 150 mg/kg of freshly prepared alloxan in distilled water. Blood glucose concentration was measured 72 h post injection, and rats with blood glucose concentration above 300 mg/dl were considered diabetic and separated for the studies.

2.8 Acute *in vivo* studies

Acute *in vivo* dose-dependent effects of CF were examined in overnight fasted non-diabetes rats. Rats received a single dose of glucose (18 mmol/kg body weight, i.p.) alone or in combination with CF (100 and 200 mg/kg bw) or 10 mg/kg bw glibenclamide (GB). The choice of doses for chloroform fraction (CF) of *A. vogelii* and GB used in this study was informed by literature (Osadebe et al., 2014; Okokon et al., 2012) and results of a pilot study conducted. Blood samples were collected prior to injection and at 15, 30, 60 and 120 min post injection for measurement of glucose concentrations.

2.9 Chronic *in vivo* studies

Normal and diabetic rats (n=6) received daily oral administration of either saline vehicle (0.9% (w/v), GB (10mg/kg bw) or CF (100 and 200 mg/kg bw) for 21 days. Body weight and non-fasting blood glucose concentration were measured at the beginning and every 7days during the study. Glucose tolerance (18 mmol/kg body weight, i.p.) was examined in all groups of rat on day 21 following an overnight fast as previously described by Irwin et al. (2006).

2.10 Biochemical Analysis

At the end of the study, blood samples were collected by cardiac puncture for measurement of serum insulin and leptin concentrations by ELISA (Insulin and Leptin DRG ELISA kits, USA), triglycerides (Tietz, 1990), total cholesterol (Trinder, 1969), high density lipoprotein cholesterol (Tietz, 1976), and low density lipoprotein cholesterol (Spectrum Diagnostics, Egypt). Insulin resistance (IR) was determined by the homeostatic model assessment (HOMA) method as described by Matthew et al (1985). $HOMA-IR = (\text{insulin } (\mu\text{IU/ml}) \times \text{glucose (mmol/l)})/22.5$. Visceral fat was isolated from within the abdominal cavity including fat around internal organs such as the kidney, liver, intestines and pancreas. Pancreases were excised for histological examination.

2.11 Histopathology

Excised pancreases were rinsed in distilled water and inserted in 10% neutral buffered formalin for histological studies. Tissues were cut into small pieces of not more than 4mm thick and inserted into pre-labelled cassettes which were further fixed by immersing in 10% formalin for 24 hours. Tissue processing and staining with haematoxylin and eosin (H&E) followed examination under light microscope (Avwioro, 2010).

2.12 Statistical Analysis

Results were presented as the Mean \pm S.E.M. One Way ANOVA followed by Tukey's Multiple Range Test was used to determine the statistical significance of difference in parameters amongst groups using GraphPad Prism 5. The significant level was taken as $P < 0.05$.

3. RESULTS

3.1 Acute toxicity study

Administration of CF (0 – 2000 mg/kg bw) to normal and diabetic rats produced no adverse effect in this study. No death of animals receiving CF was also observed in this study. These observations are indicative of lack of acute toxicity for the chloroform fraction of *A. vogelii* and provides justification for the dose of CF used in this study.

3.2 Chromatographic analysis of CF

LCMS/MS chromatographic profile of CF from *A. vogelii* revealed 23 compounds via the positive mode (Figure 1A, Table 1). These include three flavonoids ((+)-maackiain 3-O-glucoside, 5,7-dihydroxy-3',4'-dimethoxy-6,8-dimethylflavone, phellodensin D), three carbohydrates (D-glucoside, galactopinitol B, quebrachitol), three terpenes (gamma-thujaplicin, loganin, emmotin A), a phenol (phlorisobutyrophenone), a phenolic acid (methylorsellinic acid, ethyl ester), two heterocyclic compounds (N-hexanoyl-L-homoserine lactone, samidin) and five lipid derivatives (C16 sphinganine, phytosphingosine, undecanedioic acid, oleamide and 3-hydroxy-3-methyl-glutaric acid).

Analysis of CF on the ESI-MS run in the negative polarity led to the identification of thirteen compounds (Figure 1B, Table 2). These include two terpenes (oleoside 11-methyl ester, sweroside), two flavonoids (apigenin 7-methyl ether 5-glucoside, 5,7-dihydroxy-3-methoxy-4'-prenyloxyflavone), two heterocyclic compounds (5-acetylamino-6-formylamino-3-methyluracil, di-alpha-furfuryl ether); an alkaloid (theobromine), a fatty acyls- lipid (alpha-bromocrotonic acid), a phenol (ferulic acid) and an acrylic acid (3-bromo-2E-acrylic acid).

3.3 Effects of CF on α -amylase and α -glucosidase activities

In the presence of CF, dose-dependent inhibition of α -amylase activities was also observed. Effects produced ranged from 67 to 89% ($P < 0.05$) observed for 1 mg/ml to 10 mg/ml of CF respectively (Table S1). Overall, CF had inhibitory activity against α -amylase with IC_{50} value of $51.60 \pm 0.92 \mu\text{g/ml}$ compared with the IC_{50} value of $18.36 \pm 0.78 \mu\text{g/ml}$ recorded for acarbose. Results obtained in this study revealed significant reduction in the activities of α -glucosidase in the presence of CF or acarbose. CF inhibited α -glucosidase activities by 71% and 82% ($P < 0.05$) at 300 $\mu\text{g/ml}$ and 1000 $\mu\text{g/ml}$ respectively (Table S3). The overall α -glucosidase inhibitory activity of CF ($IC_{50} = 5.86 \pm 0.97 \mu\text{g/ml}$) was comparable to acarbose ($IC_{50} = 5.63 \pm 1.53 \mu\text{g/ml}$), the standard α -glucosidase inhibitor.

3.4 Acute effects of CF on glucose tolerance in normal rats

Similar trend of glucose tolerance was observed in normal rats injected with glucose alone or in combination with GB (10mg/kg bw) or CF (100 - 200 mg/kg bw) in this study (Figure 2A). Overall effects of glucose, GB or CF administration on plasma glucose concentration, presented as area under the curve (AUC 0-120), revealed similar plasma glucose levels in all groups of animals (Figure 2B).

3.5 Sub-chronic effects of CF on body weight, non-fasting blood glucose and glucose in diabetic rats

Average body weight of untreated diabetic rats decreased by 187.4% ($P < 0.001$) compared with non-diabetic rats (Figure 3A). Treatment with GB (10mg/kg bw) significantly reduced this diabetes-induced weight loss to 64.8% ($P < 0.05$). A similar trend was observed in diabetic-rats treated with CF for 21 days with observed weight loss of 42.1% ($P < 0.05$) and 47.8% ($P < 0.05$) recorded at CF concentration of 100mg/kg bw and 200mg/kg bw respectively compared with

non-diabetic rats. Compared with untreated diabetic rats, CF prevented weight loss by 35% (100mg/kg bw, $P<0.05$) and 26% (200mg/kg bw, $P<0.05$, Figure 3A). Compared with normal rats, significant elevation ($P<0.001$) of non-fasting blood glucose levels in untreated diabetic rats was observed in this study. Gradual reduction of non-fasting blood glucose levels was observed in GB treated rats over the period of the experiment (Figure 3B) and the final blood glucose at day 21 was similar to the value obtained for normal rats. However, a sharp reduction in non-fasting blood glucose was observed in diabetic rats treated with CF as from day 7 of treatment (Figure 3B).

Treatment with CF (100 – 200 mg/kg bw) for 21 days significantly improved i.p. glucose (18 mmol/kg bw) compared to diabetic control rats (Figure 3C, 3D). Compared with rats treated with GB (10mg/kg bw) which exhibited an initial rise in blood glucose followed by a sharp reduction within 30 mins of administration, the initial sharp rise in blood glucose was prevented in CF-treated rats. This was followed by a gradual reduction of blood glucose over 120 min (Figure 3C). Moreover, faster glucose clearance was also observed in CF-treated rats compared with non-diabetic rats. Integrated response of CF-treated rats to glucose challenge (AUC₁₂₀, Figure 3D) indicated a 128% ($P<0.001$) and 146% ($P<0.001$) improvement in glucose tolerance compared with untreated diabetic rats. These values are similar to improvement of 132% ($P<0.001$) observed in rats treated with GB (10mg/kg bw).

3.6 Effects of CF on visceral fat and lipid profile in diabetic rats

Visceral fat of diabetic control rats were significantly ($P<0.05$) decreased compared to the non-diabetic control and the treated groups (Table 3). Visceral fat of rats treated with CF (100 and 200 mg/kg bw) and GB (10mg/kg bw) were significantly ($P<0.05$) decreased compared to the non-diabetic control. Moreover, rats treated with CF (100 and 200 mg/kg bw) and GB (10mg/kg

bw) exhibited significantly ($P<0.05$) decreased in TG, TC and LDL levels, but significantly ($P<0.05$) increased HDL levels compared to the diabetic and non-diabetic controls.

3.7 Effects of CF-treatment on HOMA-IR, insulin and leptin concentrations in diabetic rats

Significant reduction in serum insulin concentration was observed in untreated diabetic rats ($P<0.05$, Figure 4A). However, serum insulin concentration in GB-treated (10mg/kg bw) rats was 1.25-fold higher compared to untreated rats. A similar increase in insulin concentration was in CF-treated rats, with 1.29-fold ($P<0.05$) and 1.36-fold ($P<0.05$) observed in rats treated with 100 mg/kg bw and 200 mg/kg bw of CF respectively. There was significant ($P<0.05$) increase in HOMA-IR in the untreated diabetic rats compared to the normal control (Figure 4B). But rats treated with CF (100 and 200mg/kg bw) and GB showed significant ($P<0.05$) decrease in HOMA-IR compared to untreated diabetic rats. The leptin concentrations of rats treated with CF (100 and 200 mg/kg bw) were significantly ($P<0.05$) decreased compared to diabetic control (Figure 4C). However, treatment with GB (10mg/kg bw) did not affect leptin concentrations compared to both controls.

3.8 Effects of CF on pancreas histology

Histology of pancreatic tissue excised from non-diabetic as well as treated and untreated diabetic rats are shown in Figures 5(A-E) under X400 magnification. Pancreas parenchyma of non-diabetic rats showed normal serous acinar and zymogenic cells containing abundant granular eosinophilic cytoplasm and normal interlobular connective tissues. There were normal compact islets of Langerhans consisting of round to oval collections of endocrine cells (Figure 5A). Pancreatic sections of the untreated diabetic rats, on the other hand showed various thickened

interlobular connective tissues and degenerated endocrine cells within their islets as well as areas indicating necrosis (Figure 5B). Pancreatic sections from glibenclamide-treated rats exhibited non-compact islets which appeared diffused without clear margin (Figure 5C). Sections from CF-treated (100 and 200 mg/kg) rats were similar to the normal rats (Figure 5B-E)); showing pancreatic sections with normal architecture, normal serous acinar and zymogenic cells containing abundant granular eosinophilic cytoplasm, normal interlobular connective tissue and compact islets consisting of round to oval collections of endocrine cells.

4.0 Discussion

The chromatographic fingerprint of CF of *A. vogelii* revealed compounds that have been reported to have antidiabetic properties, such as quebrachitol (QCT), loganin, sweroside, oleoside 11-methyl ester and ferulic acid. Wang et al. (2017) suggested the use of quebrachitol as potential antidiabetic agents due to its reported potent glucose-lowering effects and its therapeutic application as a β -glucosidase inhibitor is being explored (Rines et al., 2016). Moreover, loganin have been reported to ameliorate hyperglycemia in db/db mice (Yamabe et al., 2010; He et al., 2016) while report of hypoglycemic effects of oleoside 11-methyl ester and ferulic acid are also available (Omar, 2010; Ibarra et al., 2009; Ohnishi et al., 2004). Sweroside has been suggested to have antidiabetic effect due to its insulin mimicking effect on the regulation of phosphoenolpyruvate carboxykinase gene expression (Huang et al., 2016). Sweroside is the only compound found in the CF which has been previously isolated from the roots of *A. vogelii* (Chapelle, 1976). Though the collective actions of these phytochemicals may partly account for the improved glucose homeostasis observed in CF-treated rats in this study, reports of the action

of many of these phytochemicals on insulin secretion, degradation and/or sensitivity, glucose tolerance or glucose uptake are generally lacking.

The observation that glucose tolerance in normal rats injected with a single dose of CF was not significantly altered taken together with the sharp reduction in non-fasting blood glucose levels in diabetic rats after day 7 suggest that the extract may be slow acting. Moreover, the fact that glucose homeostasis is not impaired in the animals used for the acute testing may mask the initial effects of CF; which may be minimal. In addition, the fact that non-diabetic rats were used for this aspect of the study may also partly explain the absence of acute effects of CF on glucose tolerance. On the hand, the result indicated that CF administration within the dose tested in this study may not lead to hypoglycaemia, which has been recognized a lethal side effect of some currently available antidiabetic drugs (Snyder et al, 2004).

Animals fed with a high fat diet has been shown to induce characteristic features of obesity and type 2 diabetes in mice (Gault et al, 2010; Winzhell and Ahre'n, 2004). Moreover, the combination of high-fat feeding with the injection of a chemical agent to induce metabolic derangements characterizing type 2 diabetes has also been previously reported (Gault et al., 2010). Animal models produced via these strategies have become a useful model for the investigation of anti-diabetic effects of new therapeutic agents (Ojo et al, 2011; Irwin et al, 2009; Abdel-Wahab et al, 2008). The success of this approach is evident in the marked reduction in body weight, significantly elevated non-fasting blood glucose and impaired glucose tolerance observed in untreated diabetic animals (Figure 3) in this study.

In this study, daily oral administration of CF for 21 days significantly improved glucose tolerance. The fact that the body weight loss was prevented in CF-treated animals and that body weights of CF-treated animals compare with what was observed for GB-treated animals suggests that CF-administration did not produce any unusual adverse effect or affect food intake. However, our study showed that prolonged treatment with CF at doses above 100 mg/kg bw may lead to hypoglycemic condition. Findings of this study corroborate previous reports of the glucose lowering actions of *A. vogelii* extracts and fractions in diabetic rats (Osadebe et al., 2014; Sunday et al., 2016; Onyekere et al., 2017) and obese rats (Anyanwu et al., 2013).

In this study, elevated serum lipids in the untreated diabetic rats was observed. Alloxan-induced diabetes in rats is characterized by hyperlipidemia, particularly elevated plasma triglycerides and cholesterol levels (Dhandapani et al., 2002). In contrast, the CF-treated and GB-treated rats revealed decreased levels of triglycerides, cholesterol and low density lipid cholesterol. These indicated beneficial actions of CF in improving lipid profile in treated diabetic rats. Positive effects of *A. vogelii* on the lipid profile status have previously been reported in obese rats (Anyanwu et al., 2013). Moreover, alloxan administration has also been reported to induce weight loss in rats (Chougale et al., 2007). The reduction of visceral fat in the diabetic rats and CF-treated rats might be a reflection of the decreased body weight experienced by those rats. Body weight loss in CF-treated rats was lesser compared to weight loss observed in diabetic rats. This indicates that CF partially prevented body weight loss as a result of its hypolipidemic and hypoglycemic effect. Apigenin 7-methyl ether 5-glucoside, a derivative of apigenin, known for its antiobesity properties (Ono and Fujimori, 2011; Guo et al., 2015), was found in CF and may have contributed to reduced visceral fat content and body weight observed in this study. Reports

of anti-diabetic effects of other phytochemical constituents of CF are lacking in literature. Therefore, it is not yet understood if they play a role in the effects observed for CF in this study.

Diabetic rats had significantly decreased insulin levels which was the reverse in the rats treated with 100 and 200 mg/kg bw of CF indicating that CF might have insulinotropic effects. Interestingly, insulin levels observed in rats treated with glibenclamide, a sulphonylurea which acts via the K_{ATP} -dependent pathway to stimulate insulin secretion, were similar to what was observed in CF-treated rats. It is known that the observed increase in serum insulin levels could result from increased insulin production, secretion or both. This strongly provides a justification for the investigation of the insulinotropic actions of CF. Ferulic acid, one of the constituents of CF identified in this study, has been previously reported to stimulate increased plasma insulin levels in diabetic mice (Jung et al., 2007). According to Jung et al. (2007), ferulic acid also regulates blood glucose levels by increasing glucokinase activity and glycogen production in the liver. However, it is not yet clear if CF possesses these activities. Previous studies have also demonstrated the ability of plant-based materials to signal through the K_{ATP} -dependent pathway and increase insulin secretion from pancreatic beta cells (Ojo and ojo, 2015). However, it is not yet clear if this pathway is involved in the actions of CF. Moreover, beneficial actions of CF may also include increase in the proliferation of pancreatic beta cells, leading to increased insulin levels in the serum. Therefore, it is important that further studies also investigate actions of CF on beta cell proliferation.

The insulin resistance index, (HOMA-IR), measures insulin sensitivity in clinical and animal studies (Sasaki et al., 2009). Since insulin resistance is a characteristic feature of obesity-diabetes (Ahmed and Aref, 2010), increased HOMA-IR values observed in the untreated diabetic rats establishes insulin resistance as the major morbidity in this model of type 2 diabetes. However,

lower HOMA-IR values were observed for the rats treated with the CF (100 and 200mg/kg bw) is indicative of the beneficial actions of the plant extract on insulin sensitivity in rats. Leptin, an adipocyte-derived hormone is usually elevated in response to increased body fat mass (Marques et al., 2016). High fat feeding produced increased serum leptin levels in non-diabetic control. However, serum leptin levels in diabetic control and CF-treated animals were significantly reduced. It is suggested that the reduced visceral fat and body weight may play a role in the observed effect. Lower leptin levels were observed in CF-treated diabetic rats compared with diabetic control rats, which is indicative of the ability of CF to improve leptin sensitivity in rats.

Histological studies of pancreas isolated from untreated diabetic rats showed areas indicating necrosis of beta cells. Beta cells destruction caused by reactive oxygen species generation is a morphological feature in alloxan-induced diabetes (Lenzen, 2008). However, the pancreatic sections of CF-treated (100 and 200 mg/kg) rats appeared to have normal architecture and compact islets. This indicated that CF might have reversed the earlier necrosis of beta cells of islets of Langerhans induced by alloxan or enhanced the regeneration of the beta cells. The reversal of necrotic actions of alloxan by natural products after the destruction of the beta cells has been previously reported (Chakravarthy et al., 1982; Singh and Gupta, 2007).

The breakdown of complex dietary carbohydrate (starch) to simple sugar (glucose) which can be easily absorbed through the small intestine is key function of α -amylase (Kotowaroo et al., 2006). The inhibition of α -amylase decreases the amount of carbohydrates that could be digested and consequently the amount of glucose that enters the bloodstream. Alpha-amylase is not the only hydrolysing enzyme of carbohydrate digestion, it is well supported by α -glucosidase, and inhibition of both enzyme is key to diabetes management (Wongsa et al., 2012). In this study, significant inhibition of α -amylase activities by CF was observed. Similarly, the plant fraction

significantly inhibited α -glucosidase activities. While these inhibitory actions may partly explain the mechanism underlying the antidiabetic effects of CF, there is a need to fully understand the nature of the inhibition. It is known that enzyme inhibitors could mimic an enzyme's normal substrate and compete for the enzyme's binding site in a reversible or irreversible way (Walsh et al, 2007). Inhibitors could also bind to enzyme-substrate complex or bind to the enzyme to reduce its activity while not preventing the binding of the normal substrate (Hostettmann et al, 2006). The exact mechanism involved in the actions of CF observed in this study is not yet understood and remains a subject for further studies. Activities α -glucosidase and α -amylase were not measured in the diabetic animals treated with CF in this study and future studies to clarify the nature of the effects of CF on these enzymes are needed.

Conclusion

This study provides scientific evidence for the beneficial actions and traditional use of *A. vogelii* in the management and treatment of diabetes in Africa. More so, it suggests that the antidiabetic properties exhibited by the chloroform fraction of *A. vogelii* may be attributed to constituents of the extract previously reported to have antidiabetic activities. Our results suggest that the chloroform fraction of *A. vogelii* might have exerted its antidiabetic properties by increasing serum insulin concentration as well as improving the sensitivity of insulin and leptin in obesity-diabetic rats.

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Authors Contribution

Nisar-ur-Rahman, O.O. Ojo, K. Rauf and E.C. Onyeneke designed and supervised the study. The enzymes studies was done in CADR, CIIT with the help of S. Zaib and J. Iqbal. The LC- MS/MS analysis of CF of *A. vogelii* was handled by G.O. Anyanwu and S. U. Khan. The animal studies was carried out by G.O. Anyanwu. Data analysis, interpretation and write-up was done by G.O. Anyanwu and O.O. Ojo. All authors participated in editing and approving the final manuscript.

Conflict of Interest

The authors have declared that there is no conflict of interest.

REFERENCES

- Abdel-Wahab, Y.H.A., Power, G. J., Flatt, P. R., Woodhams, D.C., Rollins-Smith, L.A., Conlon, J.M., 2008. A peptide of the phylloseptin family from the skin of the frog *Hylomantis lemur* (Phyllomedusinae) with potent *in vitro* and *in vivo* insulin-releasing activity. *Peptides* 29, 2136-2143.
- Abuh FY, Wambebe C, Rai PP and Sokomba EN (1990). Hypoglycaemic activity of *Anthocleista vogelii* (Planch) aqueous extract in rodents. *Phytother. Res.* 4(1): 20-24.
- Alberti, K.G.M.M., Zimmet, P.Z., 1998. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO Consultation. *Diabet. Med.* 15, 539–553.
- Anyanwu, G.O., Onyeneke, E.C., Usunobun, U., Adegbeji, A.J. (2013). Impact of *Anthocleista vogelii* root bark ethanolic extract on weight reduction in high carbohydrate diet induced obesity in male wistar rats. *Afr. J. Biochem. Res.* 7(11), 225-232.
- Avwioro, O.G., 2010. *Histochemistry and tissue pathology, principle and techniques*, Claverianum press, Ibadan, Nigeria.

Bailey, C. J., Day, C. (2004). Metformin: its botanical background. *Practical diabetes* 21(3), 115-117.

Chakravarthy, B.K., Gupta, S, Gode, K.D., 1982. Functional beta cell regeneration in the islets of pancreas in alloxan induced diabetic rats by (-)-epicatechin. *Life Sci.* 31(24), 2693-2697.

Chapelle, J.P., 1976. Vogeloside and secologanic acid, secoiridoid glucosides from *Anthocleista vogelii* (author's transl). *Planta Med.* 29, 268–274.

Chougale, A.D., Panaskar, S.N., Gurao, P.M., Arvindekar, A.U., 2007. Optimization of alloxan dose is essential to induce stable diabetes for prolonged period. *Asian J Biochem.* 2(6), 402-408.

Dhandapani, S., Subramanian, V.R., Rajagopal, S., Namasivayam, N., 2002. Hypolipidemic effect of *Cuminum cyminum* L. on alloxan-induced diabetic rats. *Pharmacol. Res.* 46(3), 251-255.

Gault, V.A., Porter, W.D., Flatt, P.R., Holscher, C., 2010. Actions of exendin-4 therapy on cognitive function and hippocampal synaptic plasticity in mice fed a high-fat diet. *Int. J. Obesity* 34, 1341-1344.

Guariguata, L., Whiting, D.R., Hambleton, I., Beagley, J., Linnenkamp, U., Shaw, J.E., 2014. Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Res. Clin. Pract.* 103, 137–149.

Guo, X., Liu, Y., Cai, S., Wang, O., Ji B., 2015. Synergistic interactions of apigenin, naringin, quercetin and emodin on inhibition of 3T3-L1 preadipocyte differentiation and pancreas lipase activity. *Obes Res Clin Pract.* 10(3), 327-39.

Ha, K.H., Kim, D.J., 2015. Trends in the Diabetes Epidemic in Korea. *Endocrinol Metab.* 30, 142-146.

He, K., Song, S., Zou, Z., Feng, M., Wang, D., Wang, Y., Li, X., Ye, X., 2016. The hypoglycemic and synergistic effect of loganin, morroniside, and ursolic acid isolated from the fruits of *Cornus officinalis*. *Phytother. Res.* 30, 283–291.

Hostettmann, K., Borloz, A., Urbain, A., Marston, A., 2006. Natural Product Inhibitors of Acetylcholinesterase. *Curr. Org. Chem.* 10 (8), 825–847.

Huang, X.J., Li, J., Mei, Z.Y., Chen, G., 2016. Gentiopicroside and sweroside from *Veratrum baillonii* Franch induce phosphorylation of Akt and suppress Pck1 expression in hepatoma cells. *Biochem Cell Biol.* 94(3), 270-8.

Ibarra, A., He, K., Bai, N., Bily, A., Chen, X., Rühl, R., Sang, S., Visen, P., Roller, M., 2009. Active compounds from *Fraxinus excelsior* L. seeds: Anti-diabetic and body weight control effects. *Planta Med.* 75 - SL50.

IDF, 2017. International Diabetes Federation (IDF) Diabetes Atlas, 8th edn. Brussels, Belgium: International Diabetes Federation, 2017. <http://www.diabetesatlas.org>

Irwin, N., McClean, P.L., Hunter, K., Flatt, P.R., 2009. Metabolic effects of sustained activation of the GLP-1 receptor alone and in combination with background GIP receptor antagonism in high fat-fed mice. *Diabetes Obes. Metab.* 11, 603–610.

Irwin, N., Green, B.D., Gault, V.A., Cassidy, R.S., O'Harte, F.P.M., Flatt, P.R., 2006. Effects on glucose homeostasis and insulin secretion of long term activation of the glucose-dependent insulinotropic polypeptide (GIP) receptor by N-AcGIP (LysPAL(37)) in normal mice, *Peptides* 27, 893-900.

Jansson, S. P. O., Fall, K., Brus, O., Magnuson, A., W€andell, P., Ostgren, C. J., Rolandsson, O., 2015. Prevalence and incidence of diabetes mellitus: a nationwide population-based pharmaco-epidemiological study in Sweden. *Diabet. Med.* 00, 000–000 (2015). DOI: 10.1111/dme.12716.

Jiofack, T., Fokunang, C., Guedje, N., Kemeuze, V., Fongnzossie, E., Nkongmeneck, B. A., Mapongmetsem, P.M., Tsabang, N., 2010. Ethnobotanical uses of medicinal plants of two ethnoecological regions of Cameroon. *Int. J. Med. Med. Sci.* 2, 60–79.

Johnson, M.H., de Mejia, E.G., Fan, J., Lila, M.A., Yousef, G.G., 2013. Anthocyanins and proanthocyanidins from blueberry–blackberry fermented beverages inhibit markers of inflammation in macrophages and carbohydrate-utilizing enzymes *in vitro*. *Mol Nutr Food Res.* 57, 1182–1197.

Jung, E.H., Kim, S.R., Hwang, I.K., Ha, T.Y., 2007. Hypoglycemic effects of a phenolic acid fraction of rice bran and ferulic acid in C57BL/KsJ-db/db Mice. *J. Agric. Food Chem.* 55(24), 9800–9804.

Kadiri AB (2009). An examination of the usage of herbal contraceptives and abortifacients in Lagos State, Nigeria. *Ethnobotanical leaflets* 1, 16.

Kotowaroo, M.I., Mahomoodally, M.F., Gurib-Fakim, A., Subratty, A.H., 2006. Screening of traditional antidiabetic medicinal plants of Mauritius for possible alpha-amylase inhibitory effects *in vitro*. *Phytother. Res.* 20, 228-231.

Lozano, R., Naghavi, M., Foreman, K., Lim, S., Shibuya, K., Aboyans, V., Abraham, J., Adair, T., Aggarwal, R., Ahn, S.Y., 2012. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet.* 380, 2095-128.

Marques, C., Meireles, M., Norberto, S., Leite, J., Freitas, J., Pestana, D., Faria, A., Calhau, C. (2016) High-fat diet-induced obesity Rat model: a comparison between Wistar and Sprague-Dawley Rat. *Adipocyte.* 5(1), 11-21.

Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F., Turner, R.C., 1985. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 28, 412-419.

Musa, A.D., Yusuf, G.O., Ojogbane, E.B., Nwodo, O.F.C., 2010. Screening of eight plants used in folkloric medicine for the treatment of typhoid fever. *J. Chem. Pharm. Res.* 2(4),7-15.

OECD, 2002. Test No. 420: Acute Oral Toxicity - Fixed Dose Procedure, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing.

Ohnishi, M., Matuo, T., Tsuno, T., Hosoda, A., Nomura, E., Taniguchi, H., Sasaki, H., Morishita, H., 2004. Antioxidant activity and hypoglycemic effect of ferulic acid in STZ-induced diabetic mice and KK-A^y mice. *BioFactors*. 21(1-4), 315-319.

Ojo, O.O. and Ojo, C.C., 2015. Insulinotropic actions of *Moringa oleifera* involves the induction of membrane depolarization and enhancement of intracellular calcium concentration. *J. Exp. Integr. Med.* 5(1), 36-41

Ojo, O.O., Abdel-Wahab, Y.H.A., Flatt, P.R., Mechkarska, M., Conlon, J.M., 2011. Tigerinin-1R: a potent, non-toxic insulin-releasing peptide isolated from the skin of the Asian frog, *Hoplobatrachus rugulosus*. *Diabetes Obes. Metab.* 13, 1114 -1122.

Ojo, O.O., Srinivasan, D.K., Owolabi, B.O., Flatt, P.R., Abdel-Wahab, Y.H.A., 2015. Beneficial effects of tigerinin-1R on glucose homeostasis and beta cell function in mice with diet-induced obesity-diabetes. *Biochimie*. 109, 18- 26.

Okokon, J.E., Antia B.S., Udobang J.A., 2012. Antidiabetic activities of ethanolic extract and fraction of *Anthocleista djalonensis*. *Asian Pac. J. Trop. Biomed.* 2012, 461-464.

Olubomehin, O.O., Abo, K.A., Ajaiyeoba, E.O., 2013. Alpha-amylase inhibitory activity of two *Anthocleista* species and *in vivo* rat model anti-diabetic activities of *Anthocleista djalonensis* extracts and fractions. *J. Ethnopharmacol.* 146(3), 811-814.

Omar, S.H., 2010. Oleuropein in Olive and its Pharmacological Effects. *Sci. Pharm.* 78, 133-154.

Onyekere, P.F., Onyeke, B.U., Udodeme, H.O., Anosike, C. 2017. Comparative anti-diabetic activities of methanol and n-hexane extracts of *Anthocleista vogelii* (Gentianaceae) stem bark in normoglycemic and alloxan-induced diabetic rats. *ARC J. Pharm. Sci.* 3(1), 1-8.

Ono, M., Fujimori, K., 2011. Antiadipogenic effect of dietary apigenin through activation of AMPK in 3T3-L1 cells. *J. Agric. Food Chem.* 59, 13346-13352.

Osadebe, P.O., Uzor, P.F., Omeje, E.O., Agbo, M.O., Obonga, W.O., 2014. Hypoglycemic activity of the extract and fractions of *Anthocleista vogelii* (Planch) stem bark. *Trop. J. Pharm. Res.* 13(9), 1437-1443.

Rines, A.K., Sharabi, K., Tavares, C.D., Puigserver, P., 2016. Nat. Rev. Drug Discov. 15, 786–804.

Sasaki, Y., Suzuki, W., Shimada, T., Iizuka, S., Nakamura, S., Nagata, M., 2009. Dose dependent development of diabetes mellitus and nonalcoholic steatohepatitis in monosodium glutamate induced obese mice. Life Sci. 85, 490–498.

Singh, N., Gupta, M., 2007. Effects of ethanolic extract of *Syzygium cumini* (Linn) seed powder on pancreatic islets of alloxan diabetic rats. Indian J. Exp. Bio. 45(10), 861-867.

Snyder, R. W., Berns, J. S., 2004. Reviews: Use of Insulin and Oral Hypoglycemic Medications in Patients with Diabetes Mellitus and Advanced Kidney Disease. Semin. Dialysis. 17, 365–370.

Sunday, R.M., Ilesanmi, O.R, Obuotor, E.M, 2016. Anti-diabetic effect of *Anthocleista vogelii* ethanolic root extract in alloxan-induced diabetic rats. Res. J. Med. Plants. 10, 79-88.

Thomas, M.C., Cooper, M.E., Zimmet, P., 2015. Changing epidemiology of type 2 diabetes mellitus and associated chronic kidney disease. Nature Reviews –Nephrology. Advance Online Publication, 1-9.

Walsh, R., Martin, E., Darvesh, S., 2007. A versatile equation to describe reversible enzyme inhibition and activation kinetics: Modeling β -galactosidase and butyrylcholinesterase. Biochim. Biophys. Acta. - General Subjects. 1770 (5), 733–746.

Wang, D., Zhang, S., Chang, Z., Kong, D., Zuo, Z., 2017. Quebrachitol: Global Status and Basic Research. Nat. Prod. Bioprospect. 7, 113–122.

Winzell, M. S., Ahren, B., 2004. The high-fat diet-fed mouse: a model for studying mechanisms and treatment of impaired glucose tolerance and type 2 diabetes. Diabetes 53, S215–S219.

Wongsa, P., Chaiwarit, J., Zamaludien, A., 2012. In vitro screening of phenolic compounds, potential inhibition against α -amylase and α -glucosidase of culinary herbs in Thailand. Food Chem. 131, 964–971.

Xiao, Z., Storms, R., Tsang, A., 2006. A quantitative starch-iodine method for measuring alpha-amylase and glucoamylase activities. Anal. Biochem. 351, 146–148.

Yamabe, N., Noh, J.S., Park, C.H., Kang, K.S., Shibahara, N., Tanaka, T., Yokozawa, T., 2010. Evaluation of loganin, iridoid glycoside from *Corni fructus*, on hepatic and renal glucolipototoxicity and inflammation in type 2 diabetic db/db mice. Eur. J. Pharmacol. 648, 179–187.

WHO, 2016. Global report on diabetes: Diabetes Mellitus – epidemiology. World Health Organization. <http://www.who.int/mediacentre/factsheets/fs312/en/>

LEGEND TO FIGURES

Figure 1: Chromatographic analysis of chloroform fraction of *A. vogelii* via positive (A) and negative polarity (B). Details of fractions designated by numbers are presented in Table 1 for A and Table 2 for B.

Figure 2: Acute effects of chloroform fraction of *A. vogelii* on glucose tolerance in non-diabetic rats expressed as line graph (A) and area under the curve (B). CF was administered to normal rats and glucose concentrations were measured prior to injection and at 15, 30, 60 and 120 min post-injection.

Figure 3: Effects of chronic treatment with chloroform fractions of *A. vogelii* on body weight (A), blood glucose levels (B) and glucose tolerance expressed as line graph (C) and area under the curve (D) in diabetic rats. Values are expressed as mean \pm SEM with n = 5. ***P<0.001, **P<0.01, *P<0.05 compared with non-diabetic control rats. $\Delta\Delta\Delta$ P<0.001, $\Delta\Delta$ P<0.01, Δ P<0.05 compared with diabetic control rats.

Figure 4: Effects of chronic treatment with chloroform fractions of *A. vogelii* on terminal serum insulin concentration (A), HOMA-IR (B) and leptin concentration (C) in diabetic rats. HOMA-IR: Homeostatic model assessment of insulin resistance. Values are expressed as mean \pm SEM with n = 5. *P<0.05 compared with non-diabetic control rats. Δ P<0.05 compared with diabetic control rats.

Figure 5: Chronic treatment with chloroform fractions of *A. vogelii* on pancreas histology in diabetic rats. Tissues were stained with haematoxylin and eosin and image was captured under light microscope with X400 magnification. A= Tissue from non-diabetic rats treated with saline, B= Tissue from diabetic rats treated with saline, C= Tissue from diabetic rats treated with GB (10mg/kg bw) while D and E are tissues from diabetic rats treated with 100 mg/kg bw and 200 mg/kg bw of CF respectively. All animals were treated for 21 days prior to tissue removal for histology.

Table 1: Compounds results of the LCMS/MS analysis (positive) of CF of *A. vogelii*

Table 2: Compounds results of the LCMS analysis (negative) of CF of *A. vogelii*

Table 3: Effects of chloroform fraction of *A. vogelii* on visceral fat and lipid profile of rats TG- Triglycerides, TC- Total cholesterol, HDLC- High density lipoprotein cholesterol, LDLC- low density lipoprotein cholesterol. Values are expressed as mean \pm SEM with n = 5. Means not sharing common letter(s) are significantly different (P < 0.05).