Dacryodes edulis (G. Don) H.J. Lam modulates glucose metabolism, cholinergic activities and Nrf2 expression, while suppressing oxidative stress and dyslipidemia in diabetic rats

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ABSTRACT

Ethnopharmacological relevance: Dacryodes edulis L. is an evergreen tree indigenous to western and eastern Africa which is utilized for nutritional and medicinal purposes. Folklorically, different parts of the tree are used in treating and managing diabetes and its complications.

Aims: The antidiabetic effect of the butanol fraction of *D. edulis* ethanol extract (BFDE) was studied in fructose-streptozotocin induced type 2 diabetic rats.

Methods: The ethanol extract was fractionated to yield the hexane, dichloromethane, ethyl acetate, butanol and aqueous fractions. The *in vitro* antidiabetic activities of the fractions were determined by their ability to inhibit α-glucosidase activity. BDFE was the most active and showed no cytotoxic effect while stimulating glucose uptake in 3T3-L1 adipocytes. Thus, selected for *in vivo* study. Diabetic rats were grouped into 4. The negative control group was administered water only, another group was treated with metformin (200 mg/kg body-weight), while the other groups were administered BDFE at 150 and 300 mg/kg bodyweight respectively. Two other groups consisting of normal rats were given water and BFDE (300 mg/kg bodyweight) respectively, with the former serving as normal control. After 6 weeks of intervention, the rats were humanely sacrificed using appropriate anaesthesia.

Results: Treatment with the fraction significantly (p < 0.05) reduced the blood glucose level of the diabetic rats, with concomitant increase in serum insulin secretion. It also caused significant (p < 0.05) elevation of reduced glutathione level, superoxide dismutase, catalase, α-amylase, and ATPase activities, with concomitant depletion in myeloperoxidase activity, NO and MDA levels of the serum and pancreas. The pancreatic morphology and β-cell function were significantly improved in BFDE-treated rats, with restoration of the pancreatic capillary networks. Treatment with BFDE significantly (p < 0.05) inhibited the activities of glycogen phosphorylase, fructose 1,6 biphosphatase, glucose 6 phosphatase, and acetylcholinesterase, while suppressing the expression of Nrf2. HPLC analysis revealed the presence of gallic acid, vanillic acid, vanillin, and (−)-epicatechin in the fraction.

Conclusion: These results portray the antidiabetic and antioxidative properties of BFDE, which may be a synergistic consequence of the identified phenolics.

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1. Introduction

The use of medicinal plants in treating and managing various diseases and ailments such as diabetes mellitus (DM), cancers, and malaria dates to time immemorial, with their folkloric uses integrated in the cultural systems and practices of their indigenous flora. These plants also contribute to the indigenous health system of most countries in the present day, with their increasing utilizations attributed to the paradigm shift from synthetic drugs to plant-based natural products (Erukainure et al., 2018). The efficacy of folkloric claims of these plants has been validated by several studies and attributed the medicinal activities to their phytochemical properties, with phenolics being amongst the most studied phytochemicals (Decker, 1995). Amongst such plants is *Dacryodes edulis* L., belonging to the *Burseraceae* family.

Commonly known as African or bush pear, *D. edulis* is an evergreen tree indigenous to western and eastern Africa where they are consumed mostly as food: singly or in combination with other foods (Erukainure et al., 2017b). In folkloric medicine, its parts are used in the treatment of DM, malaria, hypertension, labor pain, retarded growth, skin diseases, leprosy, oral and ear conditions, and epilepsy (Ajibesin, 2011; Conrad and Uche, 2013). The antidiabetic properties of the fruits have been demonstrated (Oboh et al., 2015; Okolo et al., 2016). The antioxidant protective effects of the leaves and fruits have been reported (Agbor et al., 2007; Conrad and Uche, 2013; Oboh et al., 2015). Also reported are its antimalarial and antimicrobial properties (Miguel et al.; Zofou et al., 2013; Zofou et al., 2011). These activities can be attributed to its identified phytochemicals which includes xanthone, isorhamnetin rhamnoside, kaur-15-ene peonidin hexosides, isoquercitrin, petunidin, hyperin, cyanidin, sitosterol, urs-12-ene-3-ol acetate ascorbic acid, 2,6-dihexadecanoate and isorhamnetin hexoside (Ella Missang et al., 2003; Erukainure et al., 2017b).

Diabetes mellitus is amongst the many diseases folklorically treated with *D. edulis*. It is characterized by exacerbated blood glucose level (hyperglycemia) arising from disorders in protein, carbohydrate and fatty acid metabolisms. These metabolic disorders have been attributed to inability of the β-cells to secrete insulin as seen in type 1 diabetes (T1D) and/or inability of the cells to utilize the insulin secreted by the pancreatic β-cells as seen in type 2 diabetes (T2D) (Erukainure et al., 2018). T2D accounts for over 90% of all diabetes, making it the most prevalent (I.D.F., 2016, 2018). It is characterized by dysfunction of the β-cell as well as insulin resistance, leading to persistent hyperglycemia and dyslipidemia (Bardini et al., 2012; Kahn, 2003). Free radicals arising from persistent hyperglycemia will lead to oxidative stress, if its production overwhelms the body's endogenous antioxidant system (Maritim et al., 2003; Tiwari et al., 2013). Oxidative stress has been recognized as a major mechanism for the pathogenesis, pathophysiology and progression of complications (micro and macro) associated with T2D such retinopathy, nephropathy, and cardiopathy (Evans et al., 2002; Giacco and Brownlee, 2010). This is evidenced by several pre-clinical and clinal investigations that used antioxidants as a therapy against T2D and its complications (Sabu and Kuttan, 2002; Tiong et al., 2013). Oxidative stress has also been reported to aggravate β-cell dysfunction and insulin secretion by triggering pancreatic β-cell apoptosis via the caspase cascade (Liadis et al., 2005; Montane et al., 2014). Exacerbated expression of the nuclear factor erythroid 2–related factor 2 (Nrf2) has also been implicated in the suppression of antioxidants at the inception of T2D (He et al., 2012; Miao et al., 2012).

Thus, the study carried out to investigate the antidiabetic properties of the butanol fraction of *D. edulis* leaves ethanol extract in T2D rats. The study also investigated the inhibitory effects of the fraction on major glucogenic, cholinergic and carbohydrate-hydrolyzing enzymes. Additionally, its ability to mitigate pancreatic oxidative injury and maintain its morphology as well as suppress exacerbated Nrf2 expressions and caspase-3 were investigated.

2. Materials and methods

2.1. Plant material

The leaves of *D. edulis* were harvested obtained from Benin City, Nigeria. They were identified and authenticated, with the assignment of the voucher number LUH: 6964 at the Department of Botany, University of Lagos, Lagos, Nigeria. A sample was deposited at the herbarium of the department. The name of the plant name was further verified at http://www.theplantlist.org (http://www.theplantlist.org/tpl1.1/search?q=Dacryodes+edulis+L.+). The leaves were air-dried, powdered and stored in air-tight Ziplock bags for further analysis.

2.2. Extraction and fractionation

About 200 g of the powdered sample was defatted with n-hexane and subjected to ethanol extraction at room temperature. The resulting ethanol extract was concentrated *in vacuo* with a Büchi Rotavapor R–II [2422A0] rotary evaporator) at 40 °C. The concentrate was dissolved in distilled water and fractionated with a separatory funnel using solvents of gradient polarity solvents to yield hexane, dichloromethane (DCM), ethyl acetate (EtOAc) and butanol (BuOH) fractions respectively. The fractions were concentrated *in vacuo*, while the aqueous residue was concentrated at 50 °C with a water bath and marked as aqueous fraction. All fractions were stored at 4 °C in respective glass vials for further studies.

2.3. Enzyme inhibitory assay

The *in vitro* antidiabetic activity of the fractions was investigated by determining their inhibitory effects on α-glucosidase activities (Oboh and Ademosun, 2011). Based on the results, the BuOH fraction was chosen for further *in vivo* studies.

2.4. Glucose uptake and cytotoxicity in 3T3-L1 adipocytes

To further ascertain the antidiabetic activity of the BuOH fraction, its ability to promote glucose uptake in 3T3-L1 pre-adipocytes were investigated. This was carried out using the method of Oyedemi et al. (2013) with slight modifications. Briefly, 3T3-L1 pre-adipocytes were seeded into 96-well plates (3000 cells/well). They were cultured with Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). To differentiate into adipocytes, the pre-adipocytes were treated with 0.1 μM insulin and 0.8 μM rosiglitazone for 48 h. After differentiation, the cells were further cultured for 48 h. The media was replaced with a non-glucose RPMI media containing 8 mM ($\simeq$ 1.3 mg/mL) glucose, 0.1% bovine serum albumin and BuOH fraction (30, 60 and 120 μg/mL). Insulin (1 μM) and water served as the positive control and vehicle control, respectively. The cells were further subjected to 1.5 h incubation in a CO_2 incubator (NÜVE EC 160, Çankaya/Ankara, Turkey) at 5% CO_2 and 37 °C. The glucose oxidase/peroxidase assay reagents were used in measuring the glucose concentration. Glucose uptake (mg/mL) was measured as the difference in glucose concentration of the medium before and after incubation. GU_{50}, which the concentration of extract (μg/mL) causing 50% glucose uptake increase relative to the effect of the control was estimated (Chukwuma et al., 2016). The cytotoxic effect of extract on differentiated 3T3-L1 adipocytes during the 1.5 h treatment period was measured using standard MTT cell viability assay protocol.

2.5. Experimental animals

Thirty-eight male albino rats (Sprague-Dawley strain; 180–200 g) were obtained from the Biomedical Research Unit (BRU), University of KwaZulu-Natal, Durban, South Africa. They were acclimatized on pelletized chows with water provided *ad libitum* for 7 days under natural photo period of 12-h light-dark cycle.
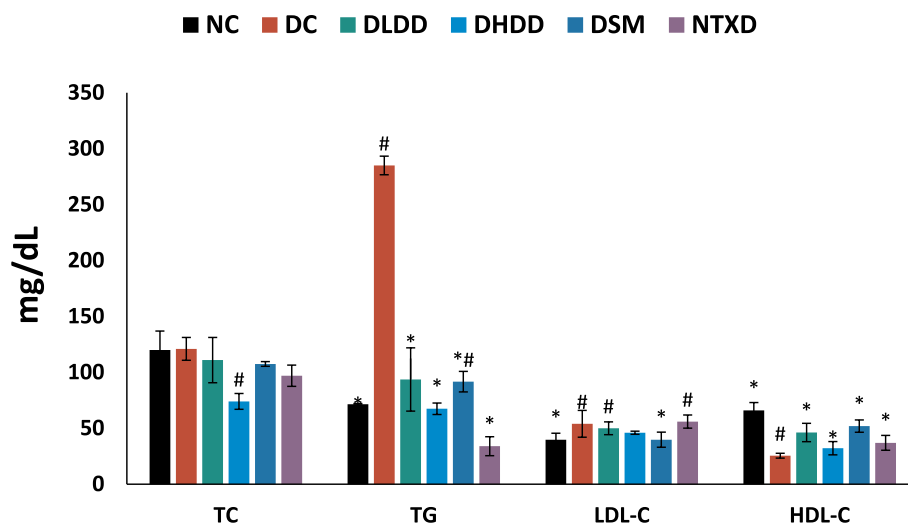
Fig. 1. α-glucosidase inhibitory activities of fractions of *D. edulis* ethanol extract. Values = mean ± SD; n = 3. [abcd]Values with different letter above the bars for a given fraction are significantly different ($p < 0.05$) from each other.

Table 1
IC$_{50}$ values of α-glucosidase inhibitory activities of fractions of *D. edulis* ethanol extract.

Fractions	IC$_{50}$ values
Hexane	50.97
DCM	164.92
Ethyl acetate	4.85
BuOH	0.13
Aqueous	97.70
Acarbose	62.42

Values = mean; n = 3.

The approved guidelines of the Animal Ethics Committee, University of KwaZulu-Natal, Durban, South Africa (protocol approval number: AREC/020/017D) were diligently followed all through the animal trials.

2.5.1. Groupings

The rats were grouped into 6 consisting of 4 groups of diabetic rats and 2 groups of normal rats. They were depicted as Normal Control (NC): Normal rats (non-diabetic); Diabetic Control (DC): Diabetic and untreated; Diabetic + low dose (DLDD): Diabetic rats treated with 150 mg/kg bodyweight [bw] of BuOH fraction of *D. edulis* (BFDE); Diabetic + high dose (DHDD): Diabetic rats treated with 300 mg/kg bw of BFDE; Diabetic + standard drug metformin (DSM): Diabetic rats treated with 200 mg/kg bw metformin; and Normal Toxicological Group (NTXD): Non-diabetic rats treated with 300 mg/kg bw of BFDE.

The non-diabetic groups (NC and NTXD) had 5 rats each, while the diabetic groups (DC, DLDD, DHDD, and DSM) had 7 rats each.

2.5.2. Induction of type 2 diabetes

Type 2 diabetes was induced using a previously established method (Wilson and Islam, 2012). Ten percent (10%) fructose were provided to the rats for 2 weeks *ad libitum*. After overnight fasting the rats induced with T2D by intraperitoneally injecting with streptozotocin (40 mg/kg BW) dissolved in citrate buffer, pH 4.5. The normal groups were injected with citrate buffer only.

After 7 days, the rats were assessed for their non-fasting blood glucose (NFBG) levels with a glucometer (Glucoplus Inc., Quebec, Canada). Animals with glucose level > 200 mg/dL were considered as diabetic. Upon diabetes induction, 26 out of the 28 rats in the diabetic groups were diabetic. The two rats belonged to the DLDD and DHDD groups and were thus excluded from the study. This implies a 93%

success rate in diabetes induction with the DLDD and DHDD groups having six animals each during the intervention period. Additionally, no rat died during the entire experimental period.

2.5.3. Intervention trial

The DLDD and DHDD groups were orally administered with BFDE at low (150 mg/kg bw) and high (300 mg/kg bw) doses respectively via gastric gavage. NTXD was administered the high dose of BFDE, while distilled water was administered to NC and DC. Daily food and fluid intake were recorded. The choice of dose was based on previous antidiabetic studies (Ibrahim et al., 2016; Mohammed et al., 2015). The blood glucose levels and body weights of the rats were monitored weekly. Intervention was carried out for 6 weeks.

2.6. Oral glucose tolerance test (OGTT)

On the last week of the intervention trial, oral glucose tolerance test (OGTT) were carried out in the rats. After an overnight fast, the rats were given a glucose solution (2 g/kg BW), orally. Their blood glucose levels were then determined at 0, 30, 60, 90 and 120 min intervals. The blood glucose level prior to administration of glucose was documented as 0 min (Erukainure et al., 2019).

2.7. Sacrifice and collection of organs

After the intervention period, the rats were euthanized with isofor after an overnight fast. Whole bloods were collected in plain tubes from each rat by cardiac puncture and placed on ice until further processing. Serums were collected after centrifuging the bloods at 4000 rpm for 10 min. They were stored at −20 °C for subsequent analysis.

Pancreas and liver were also collected from each rat. They were washed in 0.9% NaCl to remove blood stains before weighing. The relative weights of the organs were estimated as percentage ratio relative to the animal bodyweight. About 0.5 g of the pancreas was fixed in 10% neutral buffered formalin for histology and immunohistochemistry. Tiny parts of the pancreas were also fixed in 2.5% glutaraldehyde for electron microscopy. About 5 mg of each of the organs were homogenized in 5 mL 50 mM phosphate buffer, pH 7.5 with 1% triton X-100. The homogenates were subjected to centrifugation at 4 °C for 10 min at 15,000 rpm. The supernatants were collected into Eppendorf tubes and stored at −20 °C for subsequent analysis.
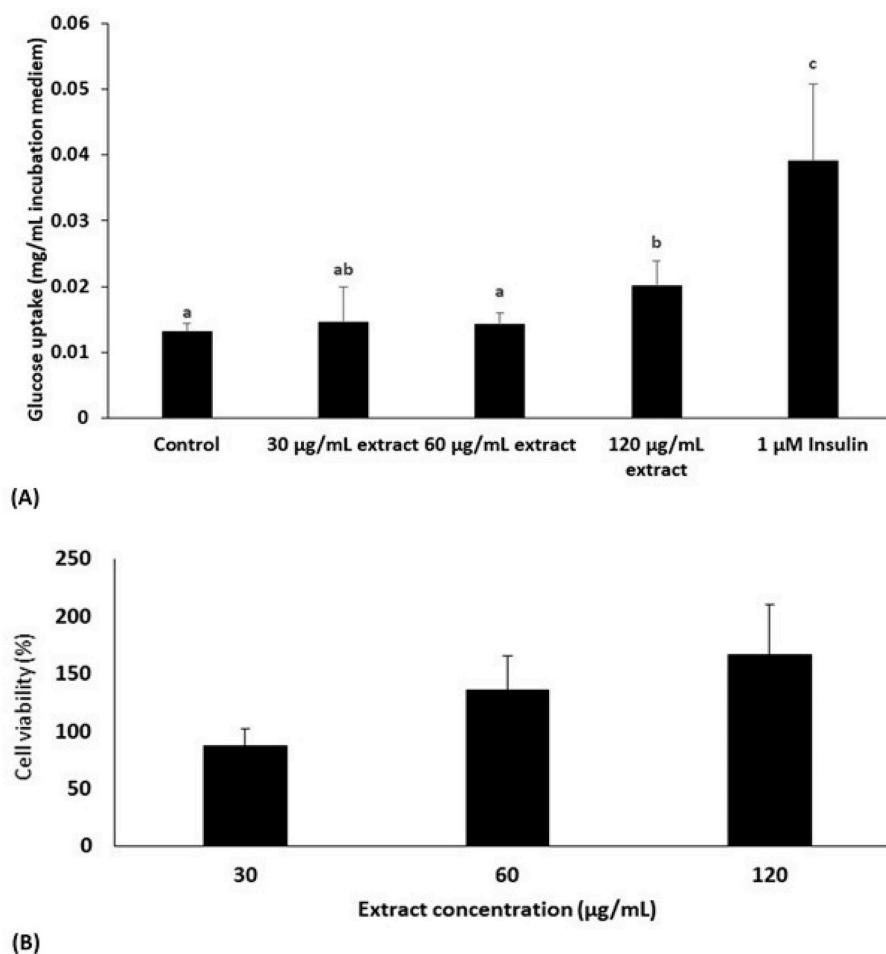
Fig. 2. (A) Effect of BFDE on glucose uptake and (B) cell viability in 3T3-L1 adipocytes. Data = mean ± SD; n = 3. [abc]Values are significantly (p < 0.05) different from each other when compared to control.

2.8. Analytical methods

Serum insulin level was determined with an ultrasensitive rat insulin ELISA kit (Mercodia, Uppsala, Sweden) using a multi-plate ELISA reader (BioTek HTX Multimode Reader, BioTek, USA). Serum levels of total protein, total cholesterol, triglycerides, and HDL-cholesterol, alanine aminotransferases (ALT), urea, alkaline phosphatase (ALP), fructosamine, uric acid, and creatine kinase–MB (CK-MB) were determined with an automated Chemistry Analyzer (Labmax Plenno, Labtest Co. Ltd., Lagoa Santa, Brazil) using commercially available assay kits as described in the manufacturer's manual.

Low-density lipoprotein (LDL) cholesterol level calculated using the Friedewald's formula (Friedewald et al., 1972) as shown below:

$$LDL - cholesterol\ (mg/dL) = Total\ cholesterol - HDL - cholesterol - \frac{Triglyceride}{5}$$

Pancreatic β-cell function and insulin resistance were computed using the homeostatic assessment models (HOMA), HOMA-β and HOMA-IR respectively as shown below:

$$HOMA - \beta\ cell\ function = \frac{360\ x\ Serum\ insulin}{Blood\ glucose\ level - 63}$$

$$HOMA - IR = \frac{Gluocose\ x\ Insulin}{405}$$

2.9. Determination of oxidative stress and proinflammatory biomarkers

Oxidative stress levels of the pancreas and blood serum were determined by analyzing for reduced glutathione (GSH) level (Ellman, 1959), catalase (Chance and Maehly, 1955) and superoxide dismutase (SOD) (Kakkar et al., 1984) activities, and malondialdehyde (MDA) level (Chowdhury and Soulsby, 2002).

Proinflammatory activities were determined by analyzing the pancreas and blood serum for myeloperoxidase activity (Granell et al., 2003) and levels of nitric oxide (NO) (Tsikas, 2005).

2.10. Determination of α-amylase activities

The pancreatic supernatants and blood serums were analyzed for their respective α-amylase activities (Oboh et al., 2017).

2.11. Determination of gluconeogenic and glycogenolytic enzymes activities

The activities of gluconeogenic and glycogenolytic enzymes were determined in the liver supernatants by assaying for glycogen phosphorylase (Balogun and Ashafa, 2017; Cornblath et al., 1963), glucose-6-phosphatase (Erukainure et al., 2017b; Mahato et al., 2011), and fructose-1,6-bisphosphatase (Balogun and Ashafa, 2017; Gancedo and Gancedo, 1971).

2.12. Determination of ATPase activity

The ATPase activities of the pancreatic supernatants and blood serums were determined using the methods described by Adewoye et al. (2000) and Erukainure et al. (2017b).
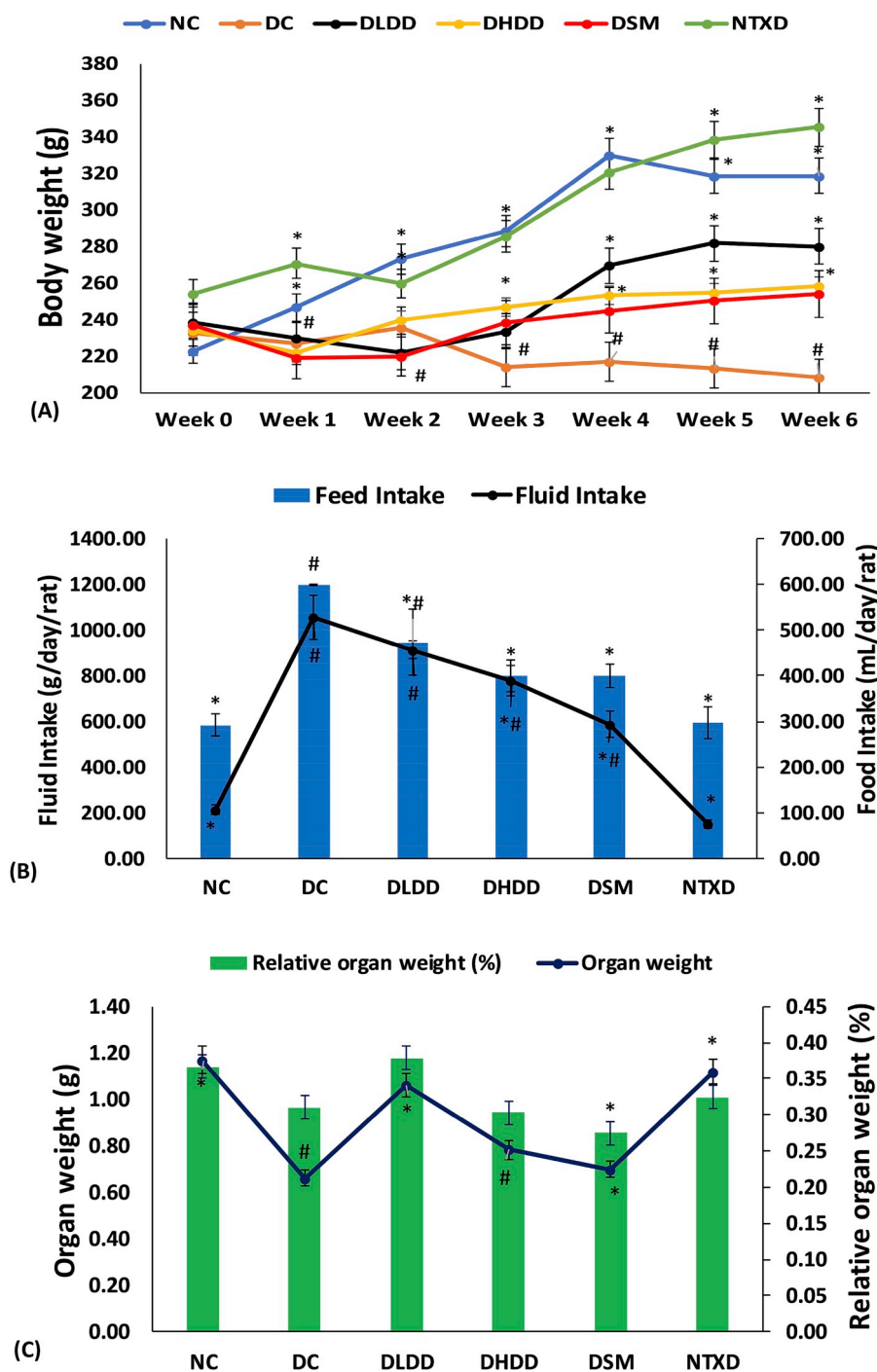
Fig. 3. (A) Body weight; (B) feed and fluid intake; and (C) weight and relative weight of pancreas of experimental groups. Values = mean ± SD; n = 5 (NC and NTXD), 6 (DLDD and DHDD) and 7 (DC and DSM). *Statistically (p < 0.05) significant to DC, #statistically significant (p < 0.05) to NC.

2.13. Determination of cholinergic activity

The cholinergic activity of the pancreatic tissues was determined by analyzing for acetylcholinesterase activity in the supernatants via the Ellman's method (Ellman et al., 1961).

2.14. Histology

After paraffin embedding, the fixed pancreatic tissues were sectioned to 4 μm on slides (Erukainure et al., 2019). They were subjected to deparaffinization and rehydration with ethanol in decreasing

concentration gradient (100%, 80%, 70%, 50%). The slides were washed with tap water before staining with hematoxylin for 5 min. They were rinsed with tap water and then stained with eosin. Images were obtained using a Leica slide scanner (SCN 4000, Leica Biosystems, Germany).

2.15. Scanning electron microscopy

The fixed pancreatic tissues were post-fixed in 0.5% osmium tetroxide for 2 h, after buffer-washing thrice at 5 min interval (Erukainure et al., 2019). They were thereafter washed thrice with distilled water at
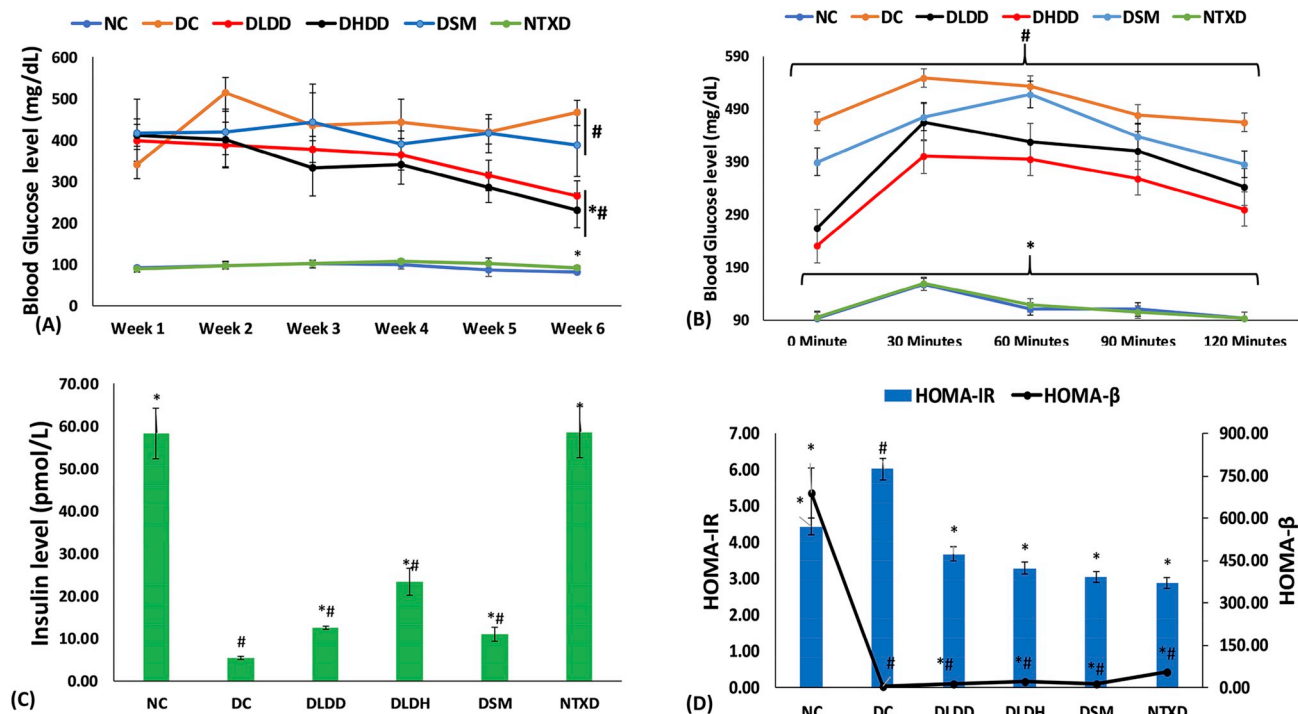
Fig. 4. (A) Blood glucose level; (B) oral glucose tolerance; (C) serum insulin; and (D) HOMA-IR and β-cell function of experimental groups. Values = mean ± SD; n = 5 (NC and NTXD), 6 (DLDD and DHDD) and 7 (DC and DSM). *Statistically ($p < 0.05$) significant to DC, #statistically significant ($p < 0.05$) to NC.
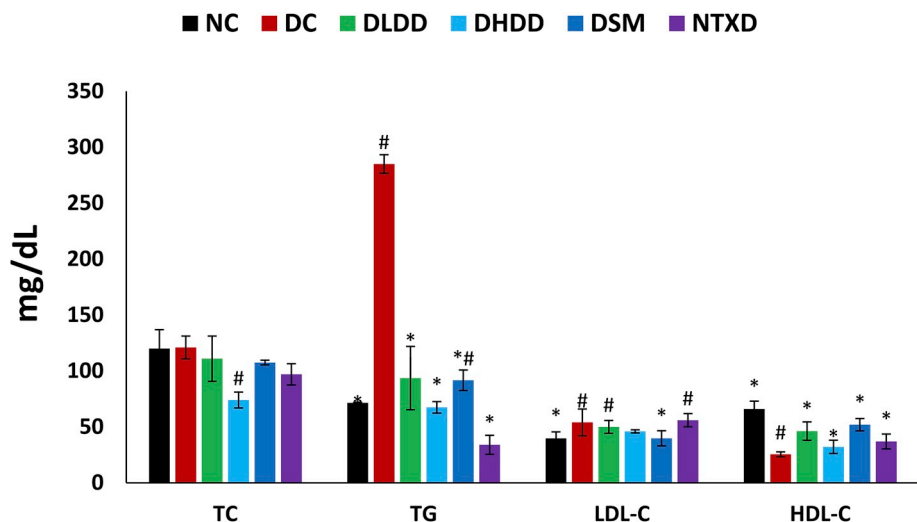

Fig. 5. Serum lipid profile of experimental groups. Values = mean ± SD; n = 5 (NC and NTXD), 6 (DLDD and DHDD) and 7 (DC and DSM). *Statistically ($p < 0.05$) significant to DC, #statistically significant ($p < 0.05$) to NC.

Table 2
Enzyme activities of experimental groups.

Enzyme Activities (U/mg protein)	NC	DC	DLDD	DHDD	DSM	NTXD
Serum α-amylase	25.38 ± 3.50*	72.36 ± 4.25#	31.21 ± 3.86*	46.41 ± 5.36#	41.57 ± 9.53*	34.11 ± 2.41*
Pancreatic α-amylase	46.90 ± 4.25*	89.72 ± 4.21#	24.32 ± 1.86*	8.42 ± 1.39*	28.02 ± 3.37*	27.29 ± 2.45*
Serum ATPase	279.18 ± 5.60*	201.24 ± 17.89#	251.38 ± 5.97*	255.70 ± 7.20*	241.92 ± 12.42*	235.30 ± 6.15*
Pancreatic ATPase	144.35 ± 21.59*	234.49 ± 7.781#	133.27 ± 15.14*	119.62 ± 25.23*	103.95 ± 14.28*	95.48 ± 13.90*#
Acetylcholinesterase	29.56 ± 3.24*	44.20 ± 3.24#	24.68 ± 1.69*	26.98 ± 1.22*	22.96 ± 3.76*	35.97 ± 3.16*
Glycogen Phosphorylase	174.22 ± 9.98*	209.89 ± 17.50#	158.36 ± 23.04*	133.54 ± 3.67*	169.35 ± 24.65*	143.32 ± 14.14*
Fructose 1,6 Biphosphatase	177.19 ± 7.93*	247.46 ± 9.90#	147.32 ± 6.80*	135.66 ± 7.07*	191.78 ± 19.49*	156.31 ± 10.57*
Glucose 6 Phosphatase	153.68 ± 3.06*	223.14 ± 12.03#	138.54 ± 9.64*	139.98 ± 5.43*	176.92 ± 23.96*	160.16 ± 13.00*

Values = mean ± SD; n = 5 (NC and NTXD), 6 (DLDD and DHDD) and 7 (DC and DSM). *Statistically ($p < 0.05$) significant to DC, #statistically significant ($p < 0.05$) to NC.
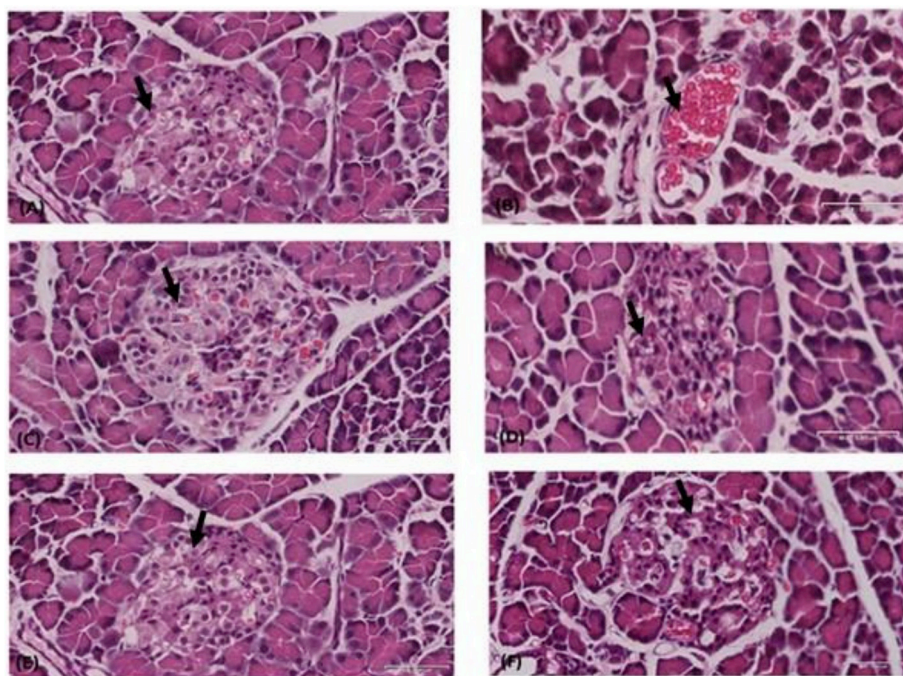
Fig. 6. Morphological changes in pancreatic tissues of experimental groups. Magnification: 10x. (A) = NC, (B) = DC, (C) = DLDD, (D) = DHDD, (E) = DSM, and (F) = NTXD. Arrow indicates β-cells.

respectively, while 1 μM metformin increased glucose uptake by 196% (Fig. 1a). Only the 120 μg/mL extract and insulin significantly increased (p < 0.05) glucose uptake, although the effect of insulin was significantly higher (p < 0.05) than that of the extract (Fig. 2A). The computed GU_{50} (concentration of extract in μg/mL causing 50% glucose uptake increase) of extract was 123.7 μg/mL. Interestingly, Fig. 1b showed that the viability of the adipocytes after the treatment period was 87, 136 and 166% at 30, 60 and 120 μg/mL extract concentration, respectively, which suggests that the extract did not markedly reduce the cells' viability after treatment. However, the proliferative tendency of the fraction on the adipocytes, particularly at 120 μg/mL, may influence its glucose uptake activity.

Induction of T2D significantly (p < 0.05) led to loss in bodyweight, with concomitant increase in food and fluid intake as depicted in Fig. 3A and B. This also led to reduced pancreatic weight and relative weight (Fig. 2C). These changes were Treatment with BFDE significantly (p < 0.05) reversed on treatment with BFDE, with rats treated with high dose showing reversibility higher than the standard antidiabetic drug, metformin.

As shown in Fig. 4A, induction of T2D significantly increased blood glucose level while causing a depletion in serum insulin level (Fig. 4C), with significantly (p < 0.05) increased HOMA- IR and reduced HOMA-β function (Fig. 4D). These were significantly (p < 0.05) reversed in all treatment groups, with BFDE treated groups showing 33.75 and 44.067% reduction in blood glucose levels for low and high doses respectively (Fig. 4A). OGTT analysis revealed an elevated blood glucose level, which peaked at 30 min for all groups except metformin treated (DSM) (Fig. 4B). The glucose level of the non-diabetic groups (NC and NTXD) were significantly (p < 0.05) lower than that of the diabetic groups (DC, DLDD, DHDD and DSM). The glucose level reduced with increasing time during the test.

Induction of T2D did not cause any significant increase in TC, but treatment with BFDE led to a significant (p < 0.05) decrease (Fig. 5). Induction of T2D however significantly (p < 0.05) increased TG and LDL-c levels, while concomitantly suppressing HDL-c level. The TG level of all treatment groups were significantly reduced to levels indistinguishable from the normal controls. Treatment with BFDE had no

significant effect on LDL-c level, while low dose of BFDE significantly (p < 0.05) increased HDL-c level.

There were significant (p < 0.05) increases in the α-amylase activities of the serum and pancreatic tissues on induction of T2D as shown in Table 2. These were significantly (p < 0.05) reduced in all treatment groups, with rats treated with low dose of BFDE showing a better inhibitory effect on serum α-amylase, while the high dosed exhibited a higher pancreatic α-amylase activity.

As shown in Table 2, there was a significant (p < 0.05) decrease in serum ATPase activity on induction of T2D. This was significantly (p < 0.05) increased in the treatment groups, with the BFDE high dose – treated rats exhibiting the highest activity.

Induction of T2D significantly (p < 0.05) increased pancreatic ATPase activity as shown in Table 1. This was significantly (p < 0.05) reversed in all treatment groups to indistinguishable levels compared to the normal controls. Rats treated with high dose of BFDE exhibited a better inhibitory effect compared to the low dose treated.

There was a significant (p < 0.05) elevated activity of the pancreatic acetylcholinesterase activity on induction of T2D as depicted in Table 1. Both doses of BFDE significantly (p < 0.05) reduced the activity to indistinguishable levels from the normal control. This was similar for the other treatment groups.

BFDE significantly (p < 0.05) inhibited T2D – induced glucogenic enzyme activities as revealed by the depleted glycogen phosphorylase, fructose-1,6-biphosphatase, and glucose-6-phosphatase activities (Table 1).

As shown in Table 2, induction of T2D caused a significant (p < 0.05) depletion in the levels of GSH, SOD, and catalase activities with concomitant elevation of MDA and NO levels, and myeloperoxidase activities. Treatment with BFDE and metformin caused a significant (p < 0.05) reversion of these levels and activities, with BFDE exhibiting the best reversal activities.

As shown in Table 3, induction of T2D caused a significant (p < 0.05) elevation of ALP, CK-MB, fructosamine and uric acid levels. These levels were significantly depleted in all treatment groups, with high dose of BFDE exhibiting reduced levels comparable to the normal control. There were no significant changes in ALT and urea levels for all groups when compared to the normal control.
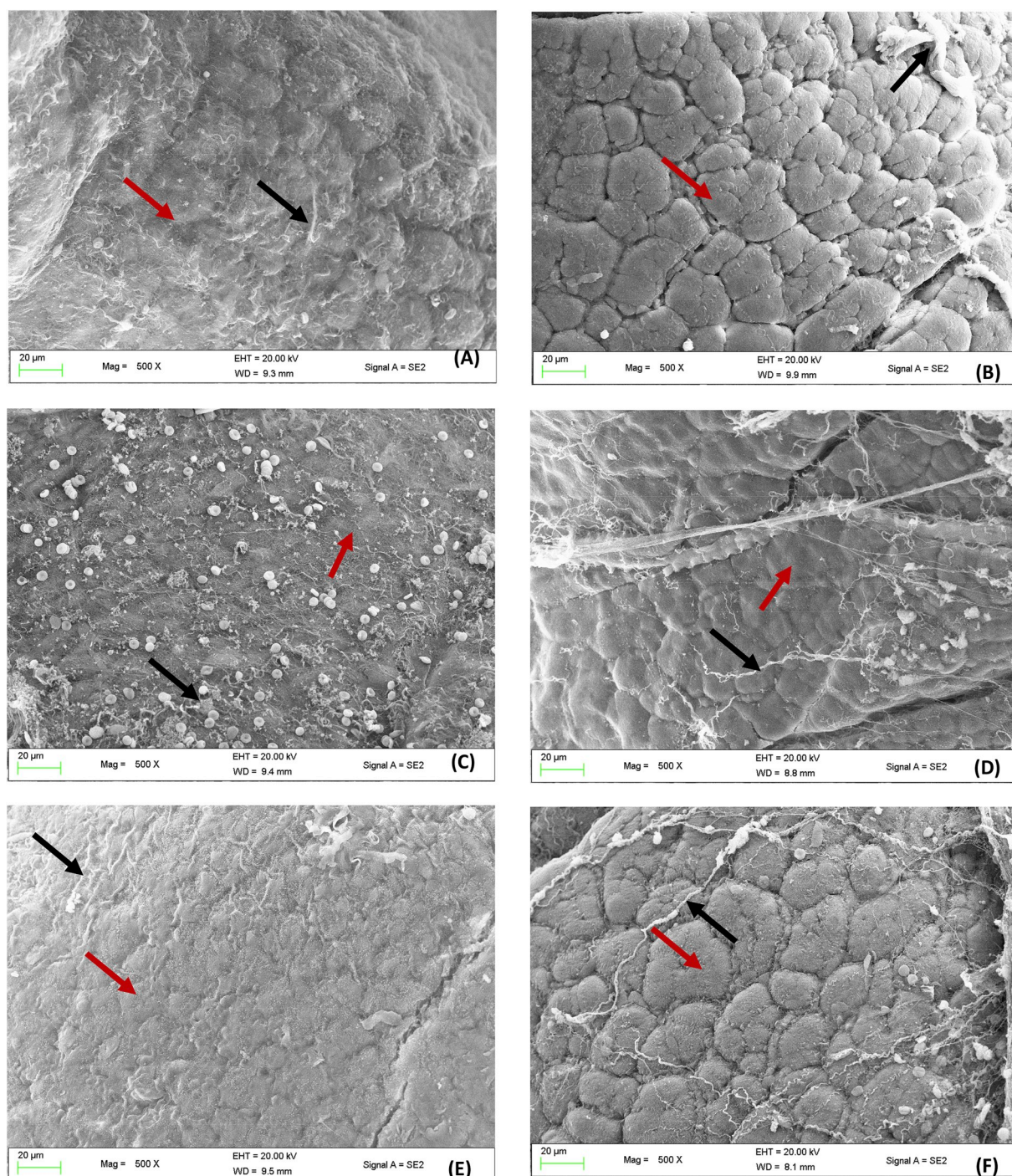
Fig. 7. Electron microscopy of pancreatic tissues of experimental groups. Magnification = 1000x. (A) = NC, (B) = DC, (C) = DLDC, (D) = DHDC, (E) = DSM, and (F) = NTXC. Arrows: Black = blood vessel; Red = acini. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

There was an alteration of the pancreatic morphology as depicted by the inflamed/necrotic β-cells (red coloured) as well as the decreased number of acinar and normal β-cells on induction of T2D as shown in Fig. 6B. The morphology was restored on treatment with BFDE as evident by the increased number of the β- and acinar cells as well as decreased number of inflamed cells (Fig. 6C and D) when compared to the intact morphology of the normal rats (Fig. 6A). This was same for all the treatment groups.

Fig. 7A *reveals* intact clusters of acini, enriched with vast networks of capillaries in pancreatic tissues of normal rats. Induction of T2D led to alteration of the capillary networks, leading to the conspicuousness of the acini clusters and enlargement of the cells as shown in Fig. 7B.

The capillary networks were restored in the treatment groups, with the low dose of BFDE exhibiting much networks (Fig. 7C–E). The acini cells were also depleted on treatment with BFDE. Administration of BFDE to normal rats led to enlarged and conspicuous pancreatic acini cells, with altered capillary networks (Fig. 7F).

As shown in Fig. 8, induction of T2D had no significant effect on pancreatic caspase-3 expression. The expressions were however increased (not significant) in BFDE treated diabetic groups (Fig. 8C and D).

Induction of T2D led to significant expression of pancreatic Nrf2 as depicted in Fig. 9. Treatment with BFDE caused a significant ($p < 0.05$) inhibition of the expression.
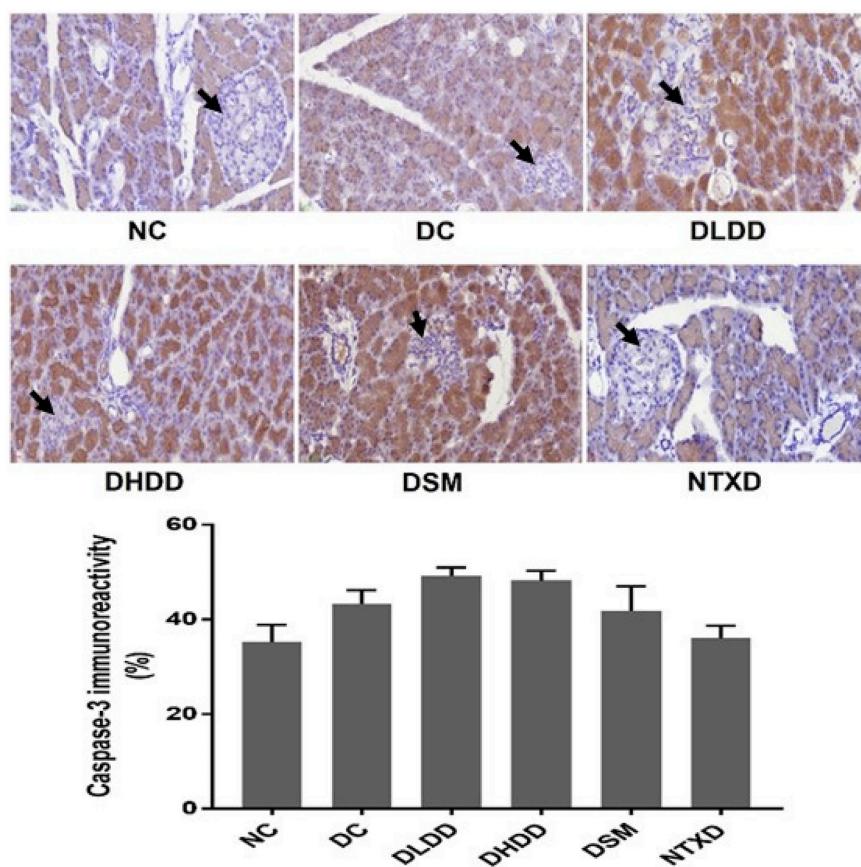
Fig. 8. Immunohistochemistry photomicrographs of caspase3 expression in pancreatic tissues of experimental groups. Magnification = 200x. Arrow indicates β – cells.

HPLC analysis of BFDE revealed the presence of gallic acid, vanillic acid, (−)-epicatechin, and vanillin as depicted in Fig. 10. (−)-epicatechin had the highest peak, indicating the highest concentration.

4. Discussion

The increasing epidemy of DM also connotes an increasing cost of its treatment, which has been reported to be an economic burden for most developing countries with little and/or poor health infrastructures. The IDF projection of 156% increase in the number people living with diabetes by 2045 in Africa (I.D.F., 2018), raises a lot of concerns on how to tackle the rising epidemic in sub-Saharan Africa. Several studies have suggested the utilization of alternative medicine in the treatment and management of DM, with particular interest in medicinal plants owing to their availability and affordability (Ekor, 2014; Mohammed et al., 2014). The antidiabetic properties of the butanol fraction of *D. edulis* ethanol extract were investigated in T2D rats.

The increased food and fluid intake, and weight loss in the untreated diabetic group (Fig. 2A and B), indicates an occurrence of polyphagia and polydipsia. Polyphagia and polydipsia as well as weight loss have been reported as key symptoms of DM (Ibrahim et al., 2016; Okon et al., 2012). Thus, the ability of BFDE to suppress these symptoms, may portray a beneficial potential in the treatment and management of T2D.

Hyperglycemia arising from pancreatic β-cell dysfunction, insulin insufficiency and resistance has been documented as the main characteristics of T2D (Abdul-Ghani et al., 2006; Cerf, 2013; Donath and Halban, 2004). This is in consent with the elevated blood glucose level (Fig. 4A), depleted serum insulin level (Fig. 4C), decreased HOMA-β value (Fig. 4D), and elevated HOMA-IR value (Fig. 4D) of the untreated diabetic rats. The depleted serum level indicates an insulin insufficiency, while the decreased HOMA-β and elevated HOMA-IR values

indicate β-cell dysfunction and insulin resistance respectively which corroborates with the elevated blood glucose level. This also correlates with the compromised pancreatic β-cell morphology (Fig. 6B) and reduced weight (Fig. 4C). Thereby indicating an occurrence of T2D. The ability of BFDE to reduce the blood glucose level, elevate serum insulin level, improve β-cell dysfunction, suppress insulin resistance, improving the pancreatic morphology and weight, demonstrates its antidiabetic potentials and affirmation of its folkloric claims. The improved glucose tolerance in diabetic rats treated with BFDE (Fig. 4B), corroborates the antidiabetic potentials of the fraction. This corresponds with previous reports on the ability of antidiabetic medicinal plants to stimulate insulin secretion and improve the pancreatic morphology, while regenerating β-cells (Erukainure et al., 2015; Ibrahim et al., 2016; Islam and Choi, 2007). This was also demonstrated in the ability of the fraction to stimulate glucose uptake in 3T3 adipocytes cells (Fig. 2A), with high a proliferative tendency (Fig. 2B).

The increased glucogenic activities which covers for glycogen phosphorylase, fructose-1,6-biphosphatase and glucose 6 phosphatase in the DC group (Table 2) indicates increased glycogenolysis, gluconeogenesis and glycolysis. This corroborates earlier reports on the elevation of these pathways in T2D (Clore et al., 2000; Guo et al., 2012). Amplification of glycogenolysis and gluconeogenesis in T2D has been implicated in the incessant glucose production from glycogen and non-carbohydrate, thereby contributing to elevated blood glucose level. Activation of these pathways therefore corroborates the elevated blood glucose level (Fig. 4A) as well as the diminished serum insulin level (Fig. 4C) in the untreated diabetic rats. The ability of BFDE to inhibit the activities of these enzymes (Table 2) may insinuate parts of its antidiabetic mechanism.

Inhibition of major dietary carbohydrate metabolizing enzymes particularly α-glucosidase and α-amylase has been recognized as a
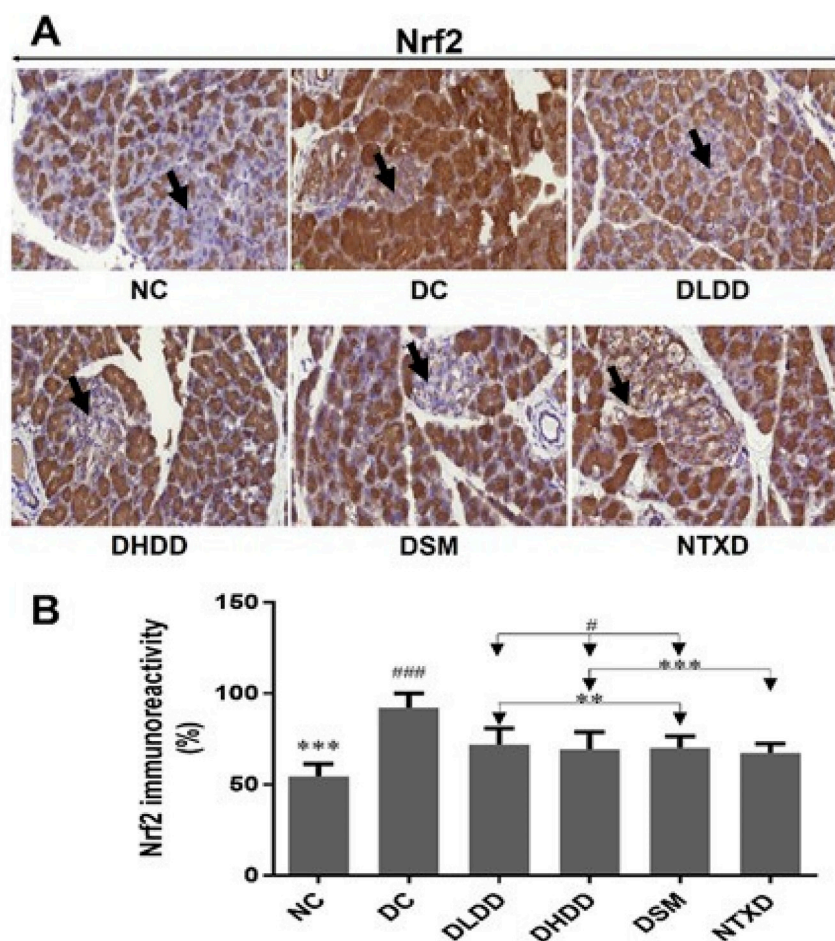
Fig. 9. (A) Immunohistochemistry photomicrographs and (B) immunoreactivity of caspase3 expression in pancreatic tissues of experimental groups. Values = mean ± SD; n = 5 (NC and NTXD) and 7 (DC, DLDD, DHDD, and DSM). *Statistically (p < 0.05) significant to DC, #statistically significant (p < 0.05) to NC. Magnification = 200x. Arrow indicates β – cells.

major antihyperglycemic mechanism which is employed by some major antidiabetic drugs like acarbose and miglitol (Chelladurai and Chinnachamy, 2018; Rahimzadeh et al., 2014). These enzymes contribute to elevated postprandial blood glucose level by breaking down dietary carbohydrate to glucose. Thus, the elevated α-amylase activities in the pancreas and serum of the untreated diabetic rats (Table 2) suggest an elevation of postprandial blood glucose level and may be a contributor to the induced hyperglycemia (Fig. 4A). This is in consent with earlier studies on amplified α-amylase activities in T2D (Aughsteen et al., 2005; Ewadh et al., 2014; Yadav et al., 2013). The distorted acinar cells (Fig. 6B) and its clusters (Fig. 7B) may also contribute to the increased α-amylase activities, as these cells functions in the secretion of digestive enzymes in the pancreas (Muniraj et al., 2015; Williams, 2010). The reduced α-amylase activities in diabetic rats treated with BFDE therefore demonstrates the antihyperglycemic activity of the fraction. This is further evidenced by their reduced blood glucose level (Fig. 4A) and improved acinar cell morphology (Fig. 6C and D) and clusters (Fig. 7C and D). This correlates with its high inhibitory potency on α-glucosidase activity *in vitro* (Fig. 1 and Table 1). An earlier study has also reported the ability of *D. edulis* leaf extracts to inhibit carbohydrate digestive enzymes *in vitro* (Erukainure et al., 2017b).

The elevated pancreatic ATPase activity in the untreated diabetic rats (Table 2) may insinuate decreased insulin secretion as studies have linked a decreased activity coupled with depolarized β – cell membrane and Ca^{2+} influx to glucose-stimulated insulin secretion (Owada et al., 1999). This may also be part of the mechanism for reduced serum insulin level (Fig. 4C). The decreased serum ATPase activity also

corroborates previous studies which reported decreased activities in T2D (Kiziltunç et al., 1997; Zadhoush et al., 2015). The reversed activities in diabetic rats treated with BFDE, thus corroborates the increased serum level (Fig. 4C) and further reflects the antidiabetic mechanism of the fraction. This correlates with earlier studies on the ability of the *D. edulis* leaf extracts to inhibit pancreatic ATPase activity (Erukainure et al., 2017b).

Disturbances in lipid metabolism has been linked with the early stage of T2D (Erukainure et al., 2013). Often described as dyslipidemia, it is depicted by increased levels of TC, TG, LDL-c, and depleted HDL-c level. Insulin resistance has been implicated in these metabolic changes which renders the lipoproteins pathogenic in T2D (Ormazabal et al., 2018). Thus, the elevated TG, LDL-c and depleted HDL-c levels in the untreated diabetic rats (Fig. 5) depicts diabetic dyslipidemia. This can be attributed to the elevated blood glucose level (Fig. 4A) and insulin resistance (Fig. 4D) on induction of T2D. The reduced TG, LDL-c and elevated HDL-c levels in diabetic rats treated with BFDE suggests an antilipemic activity of the fraction.

Oxidative stress and proinflammation have been associated with hyperglycemia-induced destruction of the pancreatic β-cells, leading to alterations in insulin sufficiency and β-cell function. This has been linked to the very low levels of the pancreatic endogenous antioxidant system, which makes it highly prone to oxidative attacks (Acharya and Ghaskadbi, 2010; Donath, 2014). The depleted GSH level, SOD and catalase activities in the untreated diabetic rats (Table 3) indicates oxidative stress. These antioxidants protect against the injurious activities of free radicals particularly superoxide anion ($O_2^{\cdot-}$). $O_2^{\cdot-}$ undergoes dismutation to hydrogen peroxide (H_2O_2) in a reaction
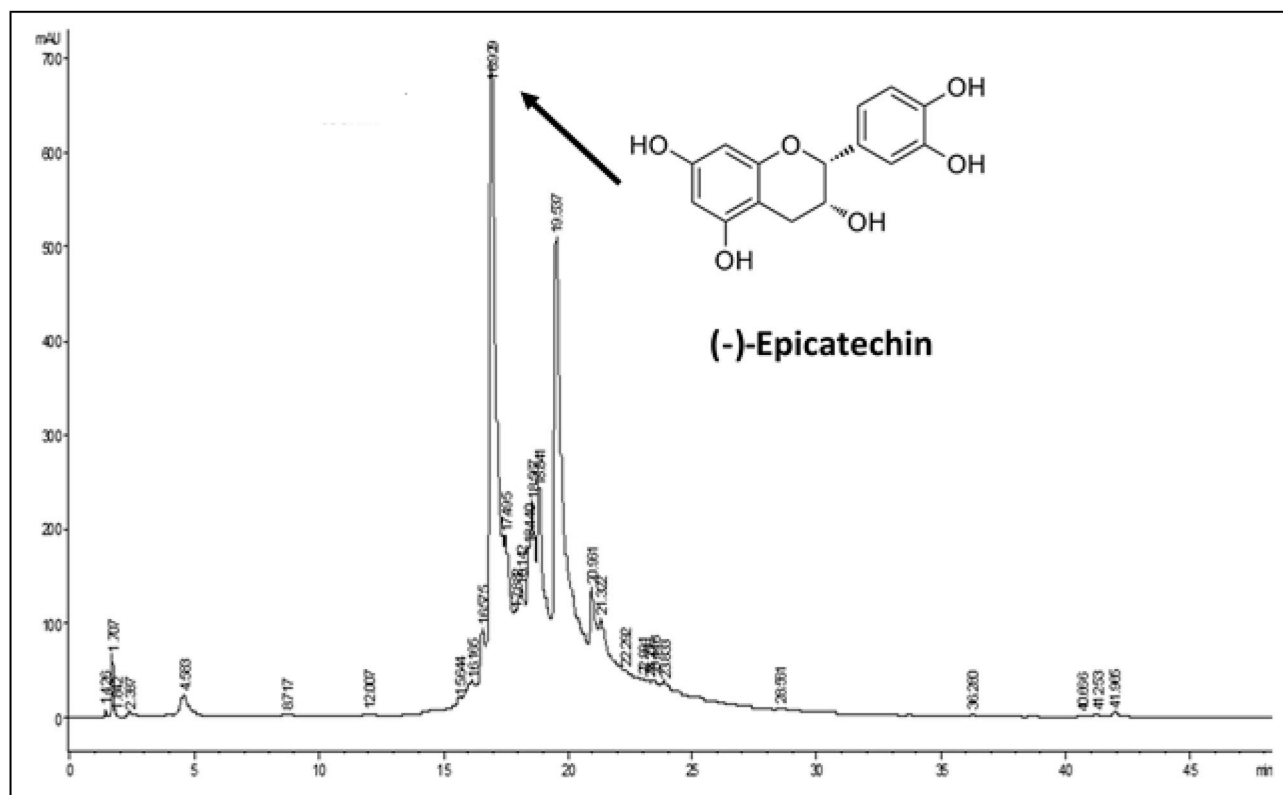
Fig. 10. HPLC chromatogram of butanol fraction of *D. edulis* ethanol extract.

Table 4
Toxicity biomarkers of experimental groups.

Enzyme Activities	NC	DC	DLDD	DHDD	DSM	NTXD
ALP U/L	101.00 ± 5.55*	1235.00 ± 29.88#	425.67 ± 63.81*	364 ± 10.80*	582.33 ± 10.67*	123.33 ± 28.99*
ALT U/L	91.00 ± 4.24	87.50 ± 0.71	88.67 ± 0.58	89 ± 8.54	88.00 ± 3.20	89.00 ± 1.00
CK-MB U/L	625.00 ± 15.80*	959.00 ± 25.10#	1104.30 ± 159.19#	727 ± 46.95*	739.40 ± 12.89#	1544.25 ± 61.97*#
Fructosamine mg/dL	656.00 ± 12.6*	743.00 ± 15.56#	698.00 ± 43.62*	605 ± 17.68*	658.50 ± 12.02*	558.00 ± 30.45*
Urea mg/dL	58.00 ± 0.71	46.00 ± 5.67	34.00 ± 5.66#	28.50 ± 4.95*#	31.50 ± 6.81#	63.33 ± 0.58*
Uric acid mg/dL	2.46 ± 0.24	3.48 ± 0.70	2.55 ± 0.68	2.07 ± 0.87	1.60 ± 0.23*#	2.75 ± 0.38

Values = mean ± SD; n = 5 (NC and NTXD), 6 (DLDD and DHDD) and 7 (DC and DSM). *Statistically ($p < 0.05$) significant to DC, #statistically significant ($p < 0.05$) to NC.

catalyzed by SOD. H_2O_2 is then broken down by catalase to water (H_2O) and O_2 (oxygen). NO can react with $O_2^{\cdot-}$ to form the lethal nitric radical, peroxynitrite ($ONOO^-$). If not converted by catalase, H_2O_2 can be broken down to hydroxyl radical (•OH) which has been recognized as a trigger for lipid peroxidation. Myeloperoxidase catalyzes the reaction of excess H_2O_2 with hydrochloric acid (HCl) to generate hypochlorous acid (HOCl). Thus, the elevated level of MDA, NO, and myeloperoxidase activity in the untreated diabetic rats (Table 2) insinuates lipid peroxidation, and proinflammation. This corroborates with the inflamed β-cells (Fig. 6B). The reversed levels and activities of these biomarkers in the BFDE treated diabetic rats, therefore insinuates an ameliorative effect of the fraction on oxidative stress and proinflammation in T2D. The latter is corroborated by the reduced acetylcholinesterase activities in BFDE-treated diabetic rats (Table 2), as increased pancreatic acetylcholinesterase activities have been correlated with inflammation of the tissue (Zhang et al., 2012).

Alteration in pancreatic perfusion have been reported in T2D, and has been implicated in β-cell dysfunction (Honka et al., 2014). Perfusion of the pancreas by multiple arteries from the abdominal aorta has been implicated in the maintenance of the pancreatic islets as well as normal physiology of the endocrine (Erukainure et al., 2019; Honka et al., 2014; Jansson et al., 2016). Thus, the altered capillary network on the pancreatic suffices of the DC group (Fig. 7B) suggests an altered perfusion. The increased networks in that of diabetic rats treated with BFDE insinuates an increased perfusion, which may also contribute to the improved β-cell function (Fig. 4D).

The detrimental effect linked with apoptosis of the pancreatic β-cells has been reported in T2D, with hyperglycemia-mediated oxidative stress and inflammation playing key roles (Acharya and Ghaskadbi, 2010; Kaneto et al., 1996; Rojas et al., 2018; Tomita, 2016). Caspase cascade has been implicated in the progression of the extrinsic apoptotic pathway (Erukainure et al., 2019), with caspase-3 reported to be very effective in β-cells apoptosis (Liadis et al., 2005; Tomita, 2016). In this study, induction of T2D had no significant effect on the expression of caspase 3 (Fig. 8), thus suggesting that the induction mechanism may not involve the caspase-3-dependent β-cell apoptosis (Erukainure et al., 2019).

The increased pancreatic Nrf2 expression in the DC group (Fig. 9B) corroborates previous studies which correlated exacerbated expressions with onset T2D (He et al., 2012; Miao et al., 2012). This also agrees with reports on its activation of oxidative stress (Wrighten et al., 2009; Zucker et al., 2014). Therefore, insinuating that the exacerbated

expression may contribute to pancreatic oxidative stress (Table 3). The decreased expressions in BFDE treated diabetic rats (Fig. 9C and D), further insinuates the antidiabetic properties of the fraction and may also be a possible antidiabetic mechanism.

The elevated level of fructosamine in the DC group (Table 4) portrays an activation of the glycation cascade, as fructosamine is an early stage glycated protein product (Erukainure et al., 2019). It is cleaved under oxidative conditions to yield the advanced glycation end (AGE) products (Ibrahim et al., 2016; Sen et al., 2011). The reduced levels in BFDE treated diabetic rats insinuates the ability of the fraction to arrest the glycation cascade.

The elevated ALP, CK-MB and uric acids levels in the DC groups (Table 4), insinuates liver, heart and kidney toxicities on induction of T2D (Erukainure et al., 2018; Mortazavi et al., 2016). Elevated levels of these toxicity biomarkers have been demonstrated in diabetic rats (Čaušević et al., 2010; Erukainure et al., 2017a; Fazel et al., 2005). Inflammation of the liver, heart and kidney has been implicated in the exacerbated levels of these biomarkers in the bloodstream (Giordano et al., 2015; Kim et al., 2008; Peppes et al., 2008). Their reduced levels in all the treatment groups as well as the non-cytotoxic effect of BFDE on 3T3-adipocytes (Fig. 2B), indicate the safety of the fraction on both diabetic and healthy tissues. This corroborates an earlier report on the predicted safety of the *D. edulis* extracts if consumed orally (Erukainure et al., 2017b).

The antidiabetic properties of BFDE may be attributed to its chemical constituents, particularly the identified phenolics consisting of gallic acid, vanillic acid, vanillin, and (−)-epicatechin (Fig. 10). As common with phenolics, the identified compounds have been reported as potent antioxidants (Makni et al., 2011; Rein et al., 2000; Rice-Evans et al., 1997) which may synergistically contribute to the antioxidative activity of the fraction in diabetic rats (Table 3). Their antidiabetic properties which encompasses improved insulin sensitivity (Josic et al., 2010), suppressed insulin resistance (Cremonini et al., 2016; Latha and Daisy, 2011), and glucose uptake (Prasad et al., 2010) have been reported. This may also contribute to the ability of the fraction to elevate serum insulin level (Fig. 4C) and suppress insulin resistance (Fig. 4D). The identified compounds may work synergistically to bring about the antidiabetic effect of the fraction.

5. Conclusion

These results insinuate the beneficial effects of BFDE in the treatment and management of T2D and its complications, as evidenced by the ability of the fraction to suppress hyperglycemia via increased insulin secretion, improved β-cell function, modulation of glucogenic and carbohydrate hydrolyzing enzymes, attenuation of hyperlipidemia, pancreatic oxidative stress, and Nrf2 expression. These may be attributed to the synergetic activities of the HPLC-identified phenolic constituents.

Authors contribution

OLE and MSI conceptualized and designed the research; OLE, XX and MSI carried out the animal study; OLE, XX and VFS carried out the biochemical assays; OMI carried out the immunochemistry assays; CIC accrued out the HPLC and cell culture analysis. OLE wrote the manuscript. All authors read and approved the submission of the manuscript.

Declaration of competing interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2020.112744.

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