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Tetracarpidium conophorum seed extract reduces intestinal absorption, and increases cellular trapping of glucose

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Abstract

Background: *Tetracarpidium conophorum* is one of the numerous folklore medicinal plants for managing diabetes but the mode of action and bioactive compounds responsible for the antihyperglycemic property are missing in literatures. This study aimed at investigating the possible modes of its antihyperglycemic action using both in-vitro and ex-vivo methods. Powdered *Tetracarpidium conophorum* seed (TECOSE) was extracted with methanol using standard extraction procedure. Gas chromatography- Mass spectrometry (GCMS) analysis of the extract, and its effects on tissue glucose uptake, α-amylase, α-glucosidase and glucokinase enzymes were assessed using standard laboratory procedures.

Results: Seven heterocyclic compounds were identified by GCMS of which one is structurally related to sulphonylurea. TECOSE strongly inhibited α-glucosidase (IC_{50} = 1.90 mg/ml) but partially inhibited α-amylase (IC_{50} = 7.20 mg/ml) activities. Also, glucokinase activity and tissue glucose uptakes were significantly ($p < 0.05$) increased by TECOSE.

Conclusions: The results obtained deduced that antihyperglycemic action of TECOSE could be due to modulation of postprandial hyperglycaemia through inhibition of intestinal α-glucosidase, increasing glucokinase activity, improving peripheral glucose uptake by mimicking sulfonylurea action.

Keywords: *Tetracarpidium conophorum* seed, Mode of action, α-Amylase, α-Glucosidase, Glucokinase

Background

Traditional medicine is gaining more acceptability for treatment of chronic ailments in several countries. In Africa, many patients rely on traditional medicine because of the high cost of the synthetic drugs. The reasons for increasing use of plants in the management of diabetes are efficacious, safety-less side effects, less expensive and easy availability of plants. Even metformin, the mainstay drug used in the treatment of type 2 diabetes, is derived from guanidines which were obtained from *Galegine officinalis* (Newman and Cragg 2012). The region of Africa has the highest percentage of undiagnosed diabetes cases reaching 66.7%, the highest proportion of diabetes mellitus related mortality and the lowest health expenditure spent on diabetes (Federation 2015). Due to adverse impact of the economic burden of diabetes, financial constraints and increased side effects of the conventional drugs, there is continuing advocacy to treat those with the disease with more affordable and accessible medicinal plant with little or no side effects. Several medicinal plants have been reported to possess antidiabetic activities (Tripathi and Chandra 2010) including the study plant. *Tetracarpidium conophorum* (African walnut) leaf, root and nut have been recently reported to possess antihyperglycemic activity (Ogbonna et al. 2013; Onwuli et al. 2014; Ajilore and Adesokan 2018; Ayeni and Nuhu 2018). Chemical compounds and mode of action responsible for antidiabetic and other therapeutic health benefits attributed to the plant are missing in literatures

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(Tiwari et al. 2011). Therefore, this study was aimed at identifying the bioactive compounds present in *T. conophorum* seed and assessing its biochemical effects on tissue glucose uptakes, α-amylase, α-glucosidase and glucokinase activities with a view to investigating its possible mode of action.

Methods

Reagents and chemicals

Methanol, concentrated H_2SO_4, acetic anhydride, ammonia solution, hydrochloric acid, citric acid, sodium citrate, streptozotocin, tris, EDTA, ATP, trichloroacetic acid, sodium chloride, sodium hydroxide, sodium hypochlorite, mono-potassium phosphate, di-potassium phosphate, ethanol, sodium carbonate, sodium acetate, acetic acid, tri-chloro acetic acid, ammonium molybdate, amino naphtol sulfonic acid, sodium bisulfate, sodium sulfite, ascorbic acid, dimethyl sulfoxide, sucrose, glucose, maltose, 3,5-dintrosalicylic acid, potassium chloride, magnesium chloride, calcium chloride, dodium bicarbonate, Humulin^R and metformin were obtained from Sigma Chemical Company, St. Lious, Mo, U.S.A., and British Drug House (BDH) chemical Ltd., Poole, England. The diagnostic kits were obtained from Randox Laboratories Ltd., Crumlin, Co. Antrim, UK. All reagents and chemicals used were of analytical grade.

Collection and preparation of Tetracarpidium conophorum seed

Tetracarpidium conophorum seeds were purchased from a local market in Osogbo, Osun State, Nigeria. The plant was identified and authenticated by Mr. G.A. Ademoriyo at Ife Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria, where specimen copy was deposited. The herbarium identification number was 17,713. The shells were removed, and the seeds shade dried for 4 weeks. The *T. conophorum* seeds were pulverized and weighed into the sample container.

Extraction of Tetracarpidium conophorum seed

Dried powder of *T. conophorum* seed (500 g) was subjected to cold maceration with frequent agitation in 5 L of 100% methanol for 72 h at room temperature (Ajilore and Adesokan 2018; Asare and Oseni 2012; Santos et al. 2018). The filtrate was concentrated using standard procedure. Methanolic extract of *T. conophorum* was stored in the fridge until used.

GC–MS analysis of Tetracarpidium conophorum seed extract

GC–MS was utilized to identify compounds in the methanol extract of the plant seed according to the method described by Santos et al. (2018).

Extraction and determination of the α-amylase activity

α-Amylase was extracted from sorghum grains according to the method described by Adewale et al. (2006) and the activity was determined spectrophotometrically at 540 nm according to the dinitro salicylic acid (DNSA) procedure of Bernfeld (1955). One unit of enzyme activity is defined as the amount of the enzyme that produces 1 µmol maltose /min under the assay conditions. Activity was calculated using enzyme activity extinction coefficient of 0.354 cm^2/mM.

Estimation of α-amylase inhibitory activity of Tetracarpidium conophorum seed

α-amylase inhibitory activity of *T. conophorum* seed extract was estimated using DNSA method as follows:

Procedure	Test (µl)	Control (µl)	Blank (µl)
Extract (1.25–10.00 mg/ml)	250	–	–
1% starch solution	500	500	–
1% NaCl	250	250	250
Phosphate buffer (0.02 M, pH 7) Pre-incubate for 5 min at 37 °C	250	250	250
α-Amylase solution Incubate again for 15 min at 37 °C	200	250	–
2 M NaOH Boil for 1 min	200	200	–
Add DNSA solution	500	500	–

The assay mixtures were incubated again for 2 min and cooled. The OD was read @ 540 nm against blank.

Calculation:

% Inhibition = (OD control − OD test)/OD control × 100.

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC50) were determined.

Mode of α-amylase inhibition

The mode of inhibition of α-amylase by *T. conophorum* seed extract was determined according to the method described by Ali et al. (2006). The amount of reducing sugar released was determined spectrophotometrically at 540 nm against blank. Concentration of maltose released from starch solution was calculated from the absorbance using maltose standard curve and converted to reaction velocities. The type of inhibition in the presence and absence of the extract on α-amylase activity was determined by analysis of the Michaelis–Menten kinetics plot.

α-Glucosidase inhibitory assay

The effect of the plant extract on α-glucosidase activity was determined according to the method described by Dahlqvist (1964). The amount of glucose liberated was measured by RANDOX commercial glucose kit.

% Inhibition Rate = (Amount of glucose produced by + ve control) − (Amount of glucose produced by addition of extract) / (Amount of glucose produced by + ve control) × 100.

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC_{50}) were determined.

Mode of α-glucosidase inhibition

The mode of inhibition of α-glucosidase by *T. conophorum* seed extract was determined according to the method described by Ali et al. (2006). The amount of glucose liberated in the presence and absence of extract was measured by RANDOX commercial glucose kit. The amount of reducing sugars released was converted to reaction velocity. The type of inhibition in the presence and absence of the extract on α-glucosidase activity was determined by analysis of the Michaelis–Menten kinetics plot.

Preparation and extraction of glucokinase eenzyme

An albino rat was fasted for 24 h after which the basal blood glucose level was determined by glucose oxidase method. The study was conducted according to the institutional guidelines and conforms to national guidelines for animal usage in research. The rat was subjected to monitored glucose tolerance test (as shown in the table below) following administration of 0.3 g/kg, 50% dextrose intra-peritoneal glucose load given over one minute.

Duration (Min)	Blood sugar (mmol/L)
0	2.89
5	6.67
10	11.11
15	25.00
20	33.33
25	35.33
30	33.06

The rat was sacrificed after 30 min and liver immediately harvested and homogenized (tissue: buffer = 1: 10) in glucokinase buffer containing 150 mM KCl, 50 mM Tris–HCl (pH 7.6), 4 mM EDTA, 4 mM Dithiothreitol and 7.5 mM $MgCl_2$ (Zhang et al. 2009). The homogenate was centrifuged at 3,000 rpm for 10 min at 4 °C following overnight lysis at 4 °C. The supernatant collected was used as enzyme extract immediately.

Assay for glucokinase enzyme activity

The effects of *T. conophorum* seed extract (TECOSE) on glucokinase activity was measured by estimating the amounts of glucose consumed and glucose-6-phosphate produced during phosphorylation of glucose in glucokinase assay mixture as follows:

Procedure	Negative control (µl)	Positive control (µl)	TECOSE (µl)
TECOSE (5–10 mg/ml)	–	–	100
Glucokinase extract	–	100	100
Glucose (100 mM and 50 mM)	100	100	100
4 mM ATP	–	100	100
7.5 mM $MgCl_2$	–	100	100

The assay mixture was incubated for 10 min at 30 °C. Glucokinase activity was calculated as mU/mg protein in the presence and absence of TECOSE as the difference between 100 and 0.5 mM glucose. Glucose concentration was determined using RANDOX commercial glucose kit while the amount of glucose-6-phosphate was measured using modified Fiske and Subbarow (1925) method by addition of ascorbic acid into the assay mixture to stabilize the phosphate ester. Protein in the liver extract was measured using RANDOX total protein kit.

Determination of glucose uptake in muscle and diaphragm

Tissue glucose uptake was determined according to the method described by Chattopadhyay (1992). Five groups, with each group containing five test tubes ($n = 5$) for each of the tissue, were considered as follows:

Group 1: Perfusion solution only (negative control).
Group 2: Perfusion solution + tissue (muscle or diaphragm).
Group 3: Perfusion solution + tissue (muscle or diaphragm) + extract.
Group 4: Perfusion solution + tissue (muscle or diaphragm) + metformin.
Group 5: Perfusion solution + tissue (muscle or diaphragm) + insulin.

Glucose concentration was determined using RANDOX commercial glucose kit.

Amount of glucose uptake by tissue = Amount of glucose in perfusate of negative control − Amount of glucose left in perfusate in other treatment groups.

Statistical analysis

Data obtained were analyzed using One Way Analysis of Variance (SPSS version 20.0). Levene statistic was used for tests of homogeneity of variance. Tukey's test was used for multiple comparisons and homogenous subsets. A p-value of less than 0.05 was considered statistically significant.

Results

Gas chromatography mass spectrometry (GCMS) analysis of Tetracarpidium conophorum seed extract

Seven aromatic compounds were identified by GCMS analysis of *T. conophorum* seed extract (Table 1 and Fig. 1).

Table 1 GCMS analysis of *Tetracarpidium conophorum* seed extract

Peak no.	Retention time (min)	Identified compounds	Other names	Molecular formula	Molecular weight (g/mol)
1	8.333	Bicyclo [4.2.0] Octa-1,3,5-triene	Benzocyclobutene	C_8H_{14}	110.2
2	36.420	Naphthalene, 1,2,3,4-tetrahydro-2- phenyl	Phenyl Tetralin	$C_{16}H_{16}$	208.3
3	36.858	Benzene, 1,1′-(1,2-cyclobutanediyl) bis-, trans	Trans-1,2-Diphenylcyclobutane	$C_{16}H_{16}$	208.3
4	42.262	Benzene, 1,1′-(3-methyl-1-propene-1,3,-diyl) bis	1-Butene, 1,3-diphenyl	$C_{16}H_{16}$	208.3
5	43.357	Benzene, 1,1′-(1,3-butadienylidene) bis	1,1-Diphenyl-buta-1,3-diene	$C_{16}H_{14}$	206.3
6	43.563	Thiocarbamic acid, *N*,*N*-dimethyl, S-1,3-diphenyl-2-butenyl ester	S-[(E)-1,3-Diphenylbut-2-enyl] *N*,*N*-dimethylcarbamothioate	$C_{19}H_{21}NOS$	311.4
	43.632				
7	43.745	Benzene, 1,1′-[2-methyl-2-(phenyl thio) cyclopropylidene] bis		$C_{22}H_{20}S$	316.5

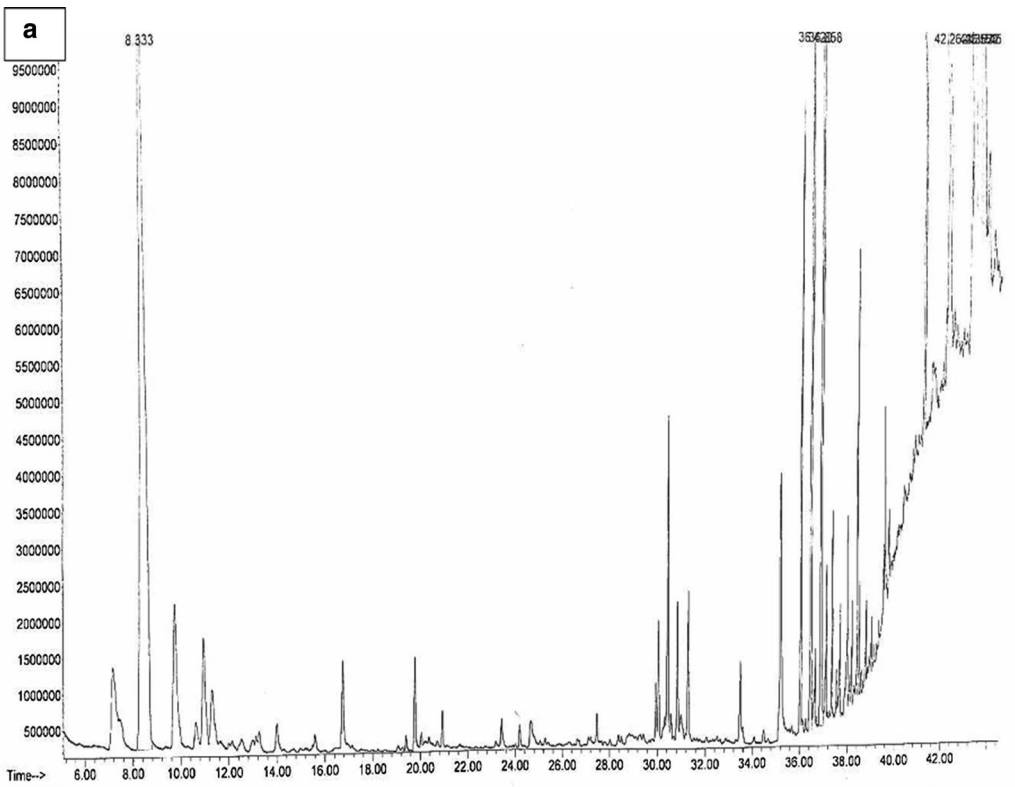

Fig. 1 GCMS analysis of *Tetracarpidium conophorum* seed extract. **a** showed spectral analysis of the methanol extract of *T. conophorum* seed while **b** showed structures of the identified heterocyclic compounds in the extract

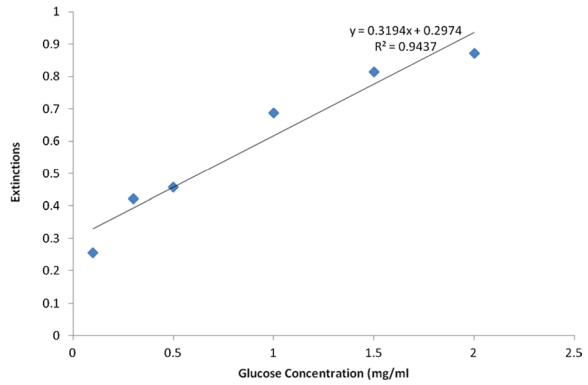

Fig. 2 Glucose standard curve

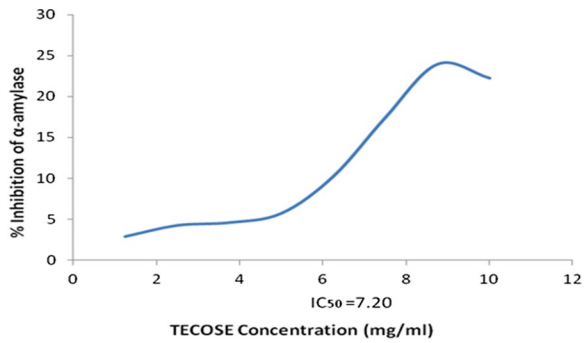

Fig. 3 Percentage inhibition of α-amylase by *Tetracarpidium conophorum* seed

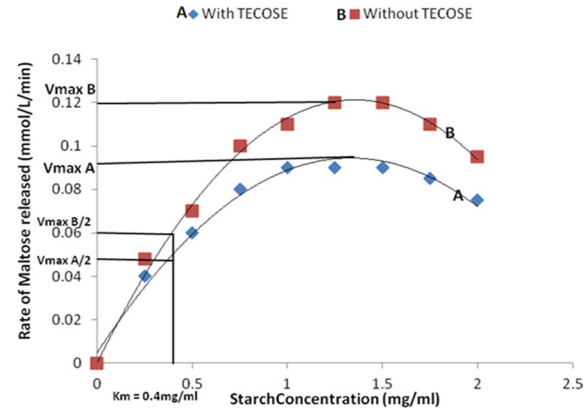

Fig. 4 Rate of maltose released from starch by α-amylase in the presence and absence of TECOSE

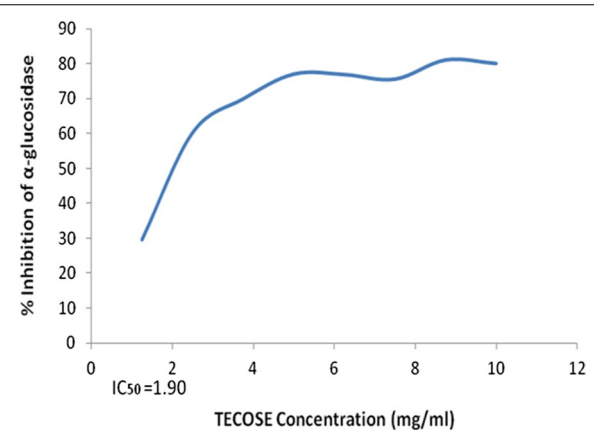

Fig. 5 Percentage inhibition of α-glucosidase by *Tetracarpidium conophorum* seed

α-Amylase and α-glucosidase activities

1 unit of α-amylase and α-glucosidase activity is defined as the amount of the enzyme required to liberate 1 mM (0.18 mg equivalence) of reducing sugar from starch and sucrose respectively under assay conditions. From the regression equation of the glucose standard curve (Fig. 2) using:

$$\text{Enzyme activity (U/ml)} = \frac{\Delta E \times Vf}{\Delta t \times \sum \times Vs \times d}$$

α-amylase activity was 0.54 U/ml while α-Glucosidase activity was 1.17 U/ml.

Inhibitory effects of Tetracarpidium conophorum seed extract (TECOSE) on α-amylase activity

The concentration of the extract that inhibited 50% of α-amylase activity (IC_{50}) was 7.20 mg/ml (Fig. 3). Maximum velocity (Vmax A) in the presence of TECOSE was 0.096 mmol/L/min and Vmax A/2 was approximately 0.048 mmol/L/min. Vmax B (in the absence of TECOSE) was 0.120 mmol/L/min and Vmax B/2 was approximately 0.060 mmol/L/min. V max decreased by the presence of TECOSE while Km (affinity) remained the same. Km was 0.400 mg/ml (Fig. 4).

Inhibitory effects of Tetracarpidium conophorum seed extract on α-glucosidae activity

The concentration of the extract that inhibited 50% of α-glucosidse activity (IC_{50}) was 1.90 mg/ml (Fig. 5). Maximum velocity (Vmax A) in the presence of TECOSE was 0.086 mmol/L/min and Vmax A/2 was approximately 0.043 mmol/L/min. Vmax B (in the absence of TECOSE) was 0.106 mmol/L/min and Vmax B/2 was approximately 0.053 mmol/L/min. V max was decreased by the presence of TECOSE while Km (affinity) remained the same. Km was 0.400 mg/ml (Fig. 6).

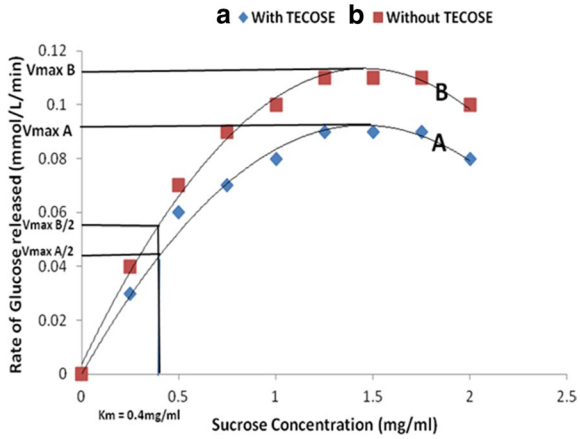

Fig. 6 Rate of glucose released from sucrose by α-glucosidase in the presence and absence of TECOSE

Table 2 Amount of glucose consumed and glucokinase activity in the presence of *Tetracarpidium* conophorum seed extract

TECOSE concentration (mg/ml)	Unused glucose (mmol/L)	Consumed Glucose (mmol/L)	Glucokinase activity (U/ml/mg protein) $\times 10^{-2}$
5.0	17.31 ± 0.03^{d}	13.94 ± 0.03^{a}	3.29 ± 0.01^{a}
10.0	9.87 ± 0.31^{c}	21.37 ± 0.31^{b}	5.05 ± 0.08^{b}
15.0	9.35 ± 1.10^{bc}	22.23 ± 0.66^{bc}	5.25 ± 0.16^{bc}
20.0	8.64 ± 0.00^{bc}	22.60 ± 0.00^{bc}	5.34 ± 0.00^{bc}
25.0	8.16 ± 1.36^{bc}	23.08 ± 1.36^{bc}	5.45 ± 0.32^{bc}
30.0	7.96 ± 0.02^{bc}	23.28 ± 0.02^{bc}	5.50 ± 0.01^{bc}
35.0	6.82 ± 2.13^{b}	24.42 ± 2.13^{c}	5.77 ± 0.50^{c}
40.0	3.53 ± 0.94^{a}	27.71 ± 0.94^{d}	6.55 ± 0.23^{d}
45.0	3.36 ± 0.64^{a}	27.89 ± 0.64^{d}	6.59 ± 0.15^{d}
50.0	2.86 ± 0.03^{a}	28.39 ± 0.03^{d}	6.71 ± 0.01^{d}

Values are expressed as mean ± SD ($n = 3$). Means with different Tukey superscripts along the column are statistically significant at $p < 0.05$

Effects of Tetracarpidium conophorum seed extract on glucokinase activity

Amount of glucose in the assay medium (negative control) was 31.24 mmol/L. Amount of glucose left in the assay medium following addition of glucokinase enzyme extract (positive control) was 30.14 mmol/L. Therefore, amount of glucose consumed by glucokinase in the absence of TECOSE, was 1.10 mmol/L (19.8 mg/dl). Concentration of total protein in liver extract was 7.62 ± 0.87 g/dl (7620 mg/dl).

Glucokinase activity (1 unit) was defined as the amount of protein (glucokinase) used in consumption of 1 mM (0.18 mg equivalence) of glucose, or 1 mM of glucose-6-phosphate produced per minute at 30 °C under the specified conditions. Therefore, estimated glucokinase activity in the absence of TECOSE was estimated to be 6.3×10^{-3} U/ml/mg protein. At increasing concentrations of TECOSE, the amounts of glucose consumed, glucose-6-phoshate produced and corresponding glucokinase activities were significantly ($p < 0.05$) increased in a dose-dependent manner (Tables 2 and 3). Glucokinase activity using the amount of glucose consumed was higher than that of glucose-6-phoshate produced at all concentrations of TECOSE. Also, glucokinase activity was significantly ($p < 0.05$) higher in the presence of TECOSE than in the absence of TECOSE (Fig. 7).

Glucose uptake by muscle and diaphragm in the control and treatment groups

Figures 8 and 9 showed the percentage glucose uptake by muscle and diaphragm respectively. The significant ($p < 0.05$) glucose uptake was in the order of metformin > insulin > TECOSE by muscle while it is metformin > TECOSE > insulin by diaphragm.

Discussion

Tetracarpidium conophorum (African walnut) was recently reported to possess antihyperglycemic property (Ajilore and Adesokan 2018; Ayeni and Nuhu 2018) but the chemical compounds or mode of action responsible for this therapeutic benefit are missing in literatures. The present study identified the bioactive compounds present in methanol extract of *T. conophorum* seed and investigated its biochemical effects on tissue glucose uptakes, α-amylase, α-glucosidase and glucokinase activities. α-glucosidase has been recognized as a therapeutic

Table 3 Amount of glucose-6-phosphate produced and glucokinase activity following incubation with *Tetracarpidium conophorum* seed extract

TECOSE concentration (mg/ml)	Glucose-6-phosphate produced (mg/dl)	Glucokinase activity (U/ml/mg protein) $\times 10^{-5}$
5.0	0.59 ± 0.01^{a}	7.68 ± 0.07^{a}
10.0	0.61 ± 0.01^{b}	7.94 ± 0.07^{b}
15.0	0.68 ± 0.01^{c}	8.86 ± 0.07^{c}
20.0	0.69 ± 0.01^{c}	8.99 ± 0.07^{c}
25.0	0.72 ± 0.01^{d}	9.39 ± 0.07^{d}
30.0	0.73 ± 0.00^{de}	9.58 ± 0.00^{de}
35.0	0.74 ± 0.01^{ef}	9.65 ± 0.07^{ef}
40.0	0.75 ± 0.01^{efg}	9.78 ± 0.07^{efg}
45.0	0.75 ± 0.00^{fg}	9.84 ± 0.00^{fg}
50.0	0.76 ± 0.01^{g}	9.97 ± 0.13^{g}

Values are expressed as mean ± SD ($n = 3$). Means with different Tukey superscripts along the column are statistically significant at $p < 0.05$

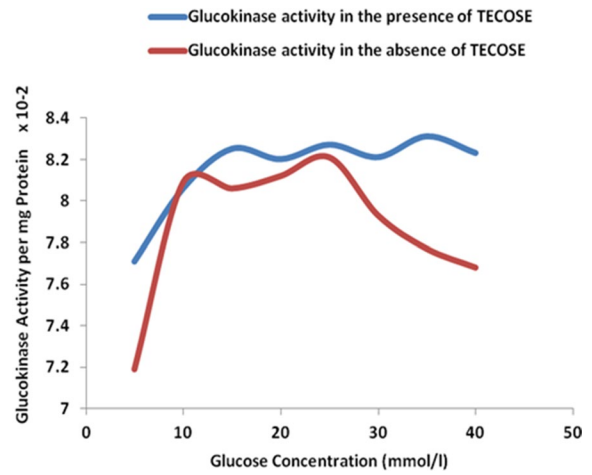

Fig. 7 Mode of glucokinase activity with and without incubation with *Tetracarpidium conophorum* seed extract

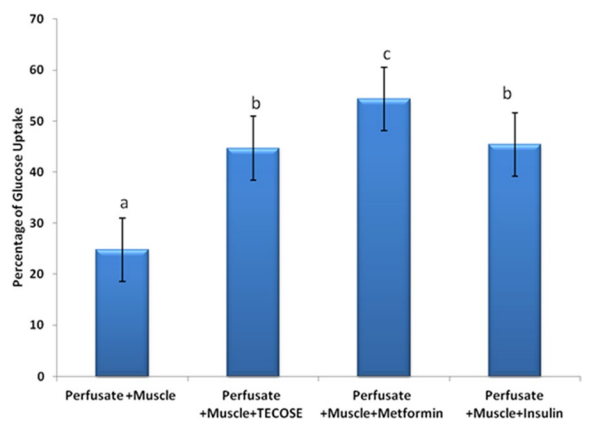

Fig. 8 Percentage glucose uptake by muscle in the control and treatment groups. Values are expressed as mean ± SD ($n = 5$). Bars with different Tukey superscripts are statistically significant at $p < 0.05$

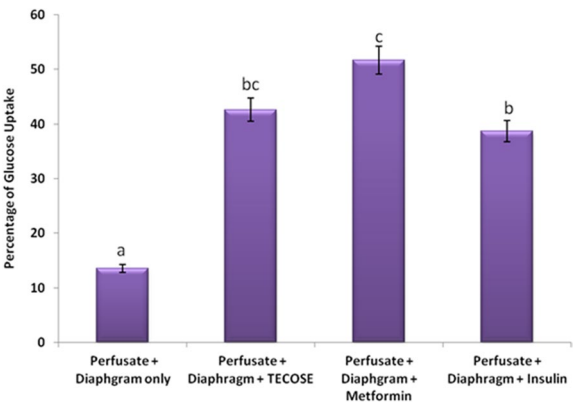

Fig. 9 Percentage glucose uptake by diaphragm in the control and treatment groups. Values are expressed as mean ± SD ($n = 5$). Bars with different Tukey superscripts are statistically significant at $p < 0.05$

target for the modulation of postprandial hyperglycemia, which is the earliest metabolic abnormality that occurs in type I diabetes (Kim et al. 2005; Thilagam et al. 2013). Therefore, an effective treatment option for type I diabetes is to inhibit the activity of intestinal α -glucosidase and pancreatic α-amylase enzymes. We observed in the present study that *T. conophorum* seed extract strongly inhibited α-glucosidase activity but demonstrated partial inhibition on α-amylase. Although α-glucosidase isolated from yeast is extensively used as a screening material for α-glucosidase inhibition, but the results did not always agree with those obtained in mammals (Thilagam et al. 2013). This was the reason rat small intestine homogenate was used as α-glucosidase solution in this study because we speculated that it would better reflect the *in-vivo* state.

Glucokinase catalyzes the transfer of phosphate from ATP to glucose to generate glucose 6-phosphate (Polonsky and Williams 2016). Liver glucokinase is rate limiting for the phosphorylation rate of glucose and is an important determinant of glucose tolerance in vivo (Stefanovski et al. 2012). The widely reported glucokinase assay in literatures is by measuring indirectly NADH/NADPH generated when Glucose-6-phosphate dehydrogenase and NAD/NADP are added into the assay mixture. The basis for this indirect assay of glucokinase, a glycolytic enzyme, using activity of glucose-6-phosphate dehydrogenase, an enzyme of another carbohydrate metabolic pathway (Hexose monophosphate shunt), is controversial and not mentioned in these literatures. In the present study, we measured glucokinase activity directly using both the amounts of glucose consumed and phosphate ester (glucose-6-phosphate) produced during phosphorylation of glucose by modifying previous methods (Zhang et al. 2009; Stefanovski et al. 2012). We observed that both the amounts of glucose consumed, and phosphate ester produced when *T. conophorum* seed extract was incubated in media containing glucokinase enzyme were significantly higher than in absence of the plant extract.

Glucose uptake by tissue plays an important role in determining glycemia. Facilitated glucose transport is essential for the maintenance of body glucose homeostasis in response to acute perturbations in blood glucose (Bryant et al. 2002; Merry and McConell 2009). Effects of treatments with *T. conophorum* seed extract, metformin and insulin on tissue glucose uptake were comparatively studied using isolated tissues (skeletal muscle and diaphragm) from normal rats. The percentage glucose uptake was significantly increased following treatments

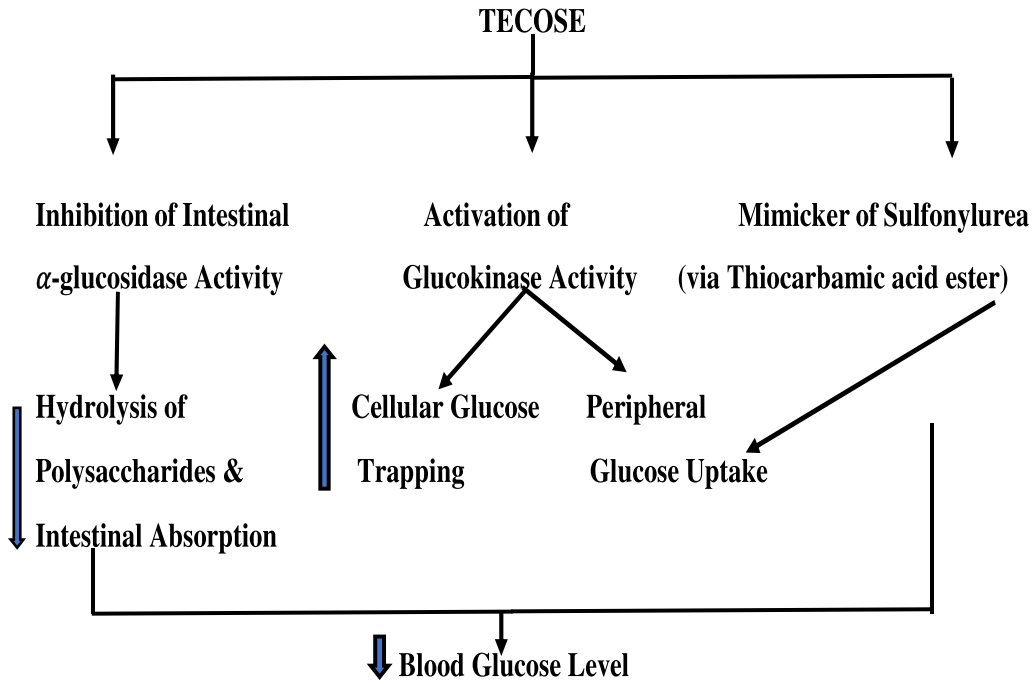

Fig. 10 Proposed mode of antihyperglycemic action of *Tetracapidium conophorum* seed extract

with metformin, *T. conophorum* seed extract and insulin. Insulin is known to stimulate uptake of glucose in fat and muscle tissues by recruiting so-called insulin-mediated glucose transporters (GLUT4) from an intracellular location to the plasma membrane (Fischer et al. 1995). Likewise, metformin is also known to increase insulin-mediated glucose uptake by improving insulin sensitivity (Iozzo et al. 2003). Mimicking these two conventional antidiabetic drugs or increasing glucokinase activity could be responsible for increased glucose uptake demonstrated by the study plant.

Beneficial effects of many plant extracts have been linked to their bioactive compounds. Seven aromatic compounds were identified from the plant seed extract. Some of the biological activities previously reported for some of the identified compounds are their uses in the management of heart-related chest pain, heart failure, depression, HIV and melanoma (Yancy et al. 2016; Aladeokin and Umukoro 2011; Kitamura et al. 2012; Bata et al. 2015; Turan-Zitouni et al. 2018). Though the biological activity for Thiocarbamic acid, *N*,*N*-dimethyl, S-1,3-diphenyl-2-butenyl ester is not found in literatures at present, but urea based compounds are known to be derivatives of carbarmic acid (Serban 2019). Sulfonylurea, a widely used antidiabetic drug, has structural relationship with Thiocarbamic acid, *N*,*N*-dimethyl, S-1,3-diphenyl-2-butenyl ester. Sulfonylurea is a sulfonyl-carbamic acid ester. Some heterocyclic- sulfonyl-carbamic acid esters have been patented in US as future antidiabetics (Hitzel et al. 1982). The *N*′,*N*′-di-phenyl- (as found in Thiocarbamic acid, *N*,*N*-dimethyl, S-1,3-diphenyl-2-butenyl ester), and others like *N*′-acetyl-, *N*′-nitro-, *N*′-cyclohexyl- could substitute the phenyl ring of sulfonylurea and could be bonded to the central S-aryl group of sulfonylurea directly or via bridge member –CH2–, –NH–, or –O–. The invention further relates that the processes of manufacture of these sulfonylureas are characterized in that -carbamic acid esters, -thiocarbamic acid esters, -ureas, -semicarbazides or -semicarbazones, which are substituted into the 4-position by the group are reacted with amine R1-NH2 or its salts or sulfonamides in the pharmaceutical preparation of these sulphonylureas for the treatment of diabetes (Hitzel et al. 1982).

Conclusions

The results obtained from this study concluded that the possible mode of action responsible for antihyperglycemic property of *Tetracarpidium conophorum* seed could be due to:

(a) Modulation of postprandial hyperglycemia through inhibition of intestinal α-glucosidase (with partial inhibition of α-amylase);
(b) Activation of glucokinase and thereby improving peripheral glucose uptake and cellular trapping;

(c) Mimicking sulfonylurea action, a known oral antidiabetic agent (Fig. 10).

Abbreviations

ATP: Adenosine triphosphate; DNSA: Dinitro salicylic acid; EDTA: Ethylenediaminetetraacetic acid; GCMS: Gas chromatography mass spectrometry; HCl: Hydrochloric acid; H_2SO_4: Sulphuric acid; IC_{50}: 50% Inhibitory capacity; MgCl: Magnesium chloride; NAD: Nicotinamide adenine di-nucleotide; NADH: Nicotinamide adenine di-nucleotide hydrogen; NADP: Nicotinamide adenine di-nucleotide phosphate; NADPH: Nicotinamide adenine di-nucleotide phosphate hydrogen; OD: Optical density; V_{max}: Maximum velocity.

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Authors' contributions

All the authors conceived and designed the study. ABS conducted the research, provided research materials and collected the data. OOS and OAO organized the data. ABS and OOS analysed and interpreted the data. ABS wrote initial and final draft of the manuscript while OOS and OAO provided logistic supports. All authors have read and approved the manuscript.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declaration

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

All authors declare no conflict of interest.

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