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ORIGINAL ARTICLE

Vernonia Amygdalina Del. stimulated glucose uptake in brain tissues enhances antioxidative activities; and modulates functional chemistry and dysregulated metabolic pathways

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Abstract

Brain glucose uptake is usually reduced in type 2 diabetes owing to downregulation of brain glucose transporters. The ability of *Vernonia amygdalina* to stimulate glucose uptake as well as ameliorate glucose-induced oxidative stress and proinflammation were investigated in rat brain. Hot infusion of *V. amygdalina* leaves was incubated with rat brain tissues for 2 h in the presence of glucose. Another incubation with glucose only, served as negative control while metformin served as positive control. Incubation of brain tissues with *V. amygdalina* led to significant ($p < 0.05$) increase in glucose uptake, reduced glutathione, nitric oxide and non-thiol proteins levels, superoxide dismutase, catalase and ATPase activities, while concomitantly decrease in myeloperoxidase activity and malondialdehyde level compared to the negative control. Incubation with glucose only, led to the development of nitrate, amide II and amide I functional groups which were removed on incubation with the infusion. LC-MS analysis revealed depletion of oxidative stress-induced 2-keto-glutaramic acid and cysteinyl-tyrosine metabolites in brain tissues, with concomitant generation of S-formylglutathione and adenosine tetraphosphate by the infusion. Pathway analysis of the metabolites revealed an activation of pyruvate metabolism pathway in the negative control, with the infusion reducing the intensity fold. LC-MS analysis of the infusion revealed the presence of l–serine, l-cysteine, l-proline, nicotinic acid, cumidine, salicylic acid, isoquinoline, 3-methyl-, and γ-octalactone. Except for l–serine, l-cysteine and l-proline, the other compounds were predicted to be permeable across the blood brain barrier. These results indicate the brain glucose uptake stimulatory and neuroprotective effect of *V. amygdalina*.

Keywords Antioxidative · Diabetic brain · Glucose uptake · Proinflammatory · *Vernonia amygdalina*

Introduction

Diabetes mellitus (DM) has been recognized as a global epidemic affecting over 425 million people in 2017, with an estimated 48% increase postulated for 2045 (I.D.F. 2018). It is a metabolic disorder characterized by high blood glucose (hyperglycemia) owing to failure of the pancreatic β cell to secrete insulin and/or inability of the cells to utilize secreted insulin as seen in type 1 diabetes (T1D) and type 2 diabetes (T2D) respectively (Erukainure et al. 2017a). T2D accounts for more than 90% of all diabetes types, thus making it a major contributor to diabetic morbidity and mortality (I.D.F. 2018). Increased production of reactive oxygen species (ROS) have been reported in extreme hyperglycemia in T2D (Erukainure et al. 2018; Maritim et al. 2003). Inability of the tissues' endogenous system to mop these free radicals, results in oxidative stress which has been implicated in the pathogenesis and progression of microvascular and macrovascular complications in T2D (Whitlow et al. 2015). Several recent studies reported that T2D is one of the major culprits in inducing microvascular complications in brain

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(Vagelatos and Eslick 2013; Whitlow et al. 2015; Wrighten et al. 2009). This has been attributed to alteration in insulin signaling and glucose homeostasis in the CNS (Wrighten et al. 2009). Glucose is the predominant source of energy in the brain and is transported across the blood brain barrier (BBB) by glucose transporters (McEwen and Reagan 2004; Reagan et al. 2008; Wrighten et al. 2009). These transporters are often down regulated in T2D, thus impairing glucose uptake in the brain (Gejl et al. 2017; Hwang et al. 2017; Pardridge et al. 1990).

The neuroprotective effects of medicinal plants have been reported in a number of recent studies (Aslam and Sial 2014; Mohebbatia et al. 2017; Pandit 2011; Uddin et al. 2013). These effects have been attributed to the phytochemical constituents of the plants, particularly the phenolics with reported antioxidant, antidiabetic and ability to transverse the BBB (Rice-Evans et al. 1997; Saravanan and Parimelazhagan 2014; Youdim et al. 2003). Some of these plants have also been reported for their antidiabetic properties, indicating their protective potential against diabetic brain degeneration. Amongst such plants is *Vernonia amygdalina*.

Vernonia amygdalina is amongst the most studied plants for its medicinal properties. It is a leafy vegetable regarded as bitter leaf owing to its bitter taste. It is consumed as a food and employed in traditional medicine in the treatment of various ailments including diabetes, hypertension, and infertility (Farombi and Owoeye 2011; Ijeh and Ejike 2011). Its folkloric medicinal claims have been authenticated by several studies which includes anti-obesogenic (Adaramoye et al. 2008), antioxidant (Adesanoye and Farombi 2010; Iwalokun et al. 2006), anticancer (Howard et al. 2003; Yedjou et al. 2008), antidiabetes (Ong et al. 2011; Saliu et al. 2012), anti-sickling (Afolabi et al. 2012; Chikezie 2006) and antihypertension (Ajibola et al. 2011; Saliu et al. 2012). Its protective effects against brain degeneration as well as in improving learning have also been reported (Ebuehi and Ajagun-Ogunleye 2017; Farombi and Owoeye 2011; Owoeye et al. 2011). Its reported phytochemical constituents include steroid glucosides, sesquiterpene lactones, stigmastane-type and steroidal saponins, terpenes, and polyphenols (Farombi and Owoeye 2011; Ijeh and Ejike 2011; Saliu et al. 2012; Yeap et al. 2010).

Although the neuroprotective effect of *V. amygdalina* has been reported, there is however a dearth in its ability to stimulate glucose uptake in brain tissues and the metabolic pathways that may be involved. Hence, this study was undertaken to investigate the glucose uptake enhancing properties of *V. amygdalina* hot infusion, and its antioxidative and anti-proinflammatory effects in brain tissues, as well as the metabolic pathways and metabolites that may be involved. The ability of d-glucose to molecularly interact with key antioxidant and proinflammatory enzymes was also investigated in silico.

Materials and methods

Plant material

Vernonia amygdalina leaves were harvested in January 2017 at Benin city, Nigeria. They were identified, deposited and assigned the voucher number: UBH_{V342} at the herbarium of the Department of Botany, University of Benin, Benin city, Nigeria.

The leaves were air dried to a constant weight, blended, and stored at room temperature in zip-lock bags until further analysis.

Extraction

Blended *V. amygdalina* leaves sample (10 g) was infused in boiling distilled water and allowed to extract overnight. The extract was decanted and concentrated in a water bath at 50 °C to yield 4.2 g concentrate, which was stored in glass vials at 4 °C until further analysis.

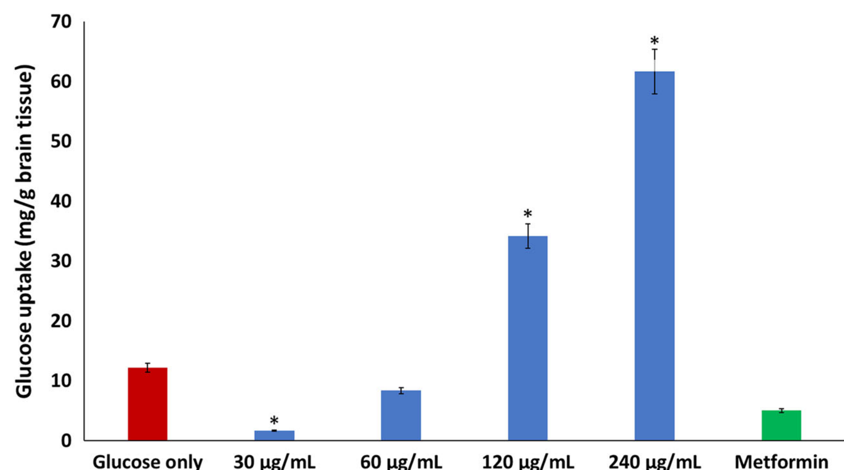

Fig. 1 Effect of *V. amygdalina* infusion on brain glucose uptake. Data = mean $\pm$ SD; $n = 3$. *Statistically significant compared to Glucose-only treated tissues

A stock solution of 1 mg/mL was prepared with distilled water. Different concentrations consisting of 15, 30, 60, 120 and 240 μg/mL were prepared from the stock solution for the study.

Animals

Five male albino rats (Sprague Dawley strain; 200–250 g) were obtained from the Biomedical Research Unit (BRU), University of KwaZulu-Natal, Durban, South Africa. They were humanely sacrificed by euthanizing with halothane after overnight fasting. Their brains were collected for ex vivo studies.

The animals were maintained in accordance with the approved guidelines of the animal ethics committee of the University of KwaZulu-Natal, Durban, South Africa (protocol approval number: AREC/020/017D).

Glucose uptake in isolated rat brain

This was carried out by incubating 0.6 g of the fresh harvested brains with 8 mL of Krebs buffer containing 11.1 mM glucose and different *V. amygdalina* infusion concentrations under a

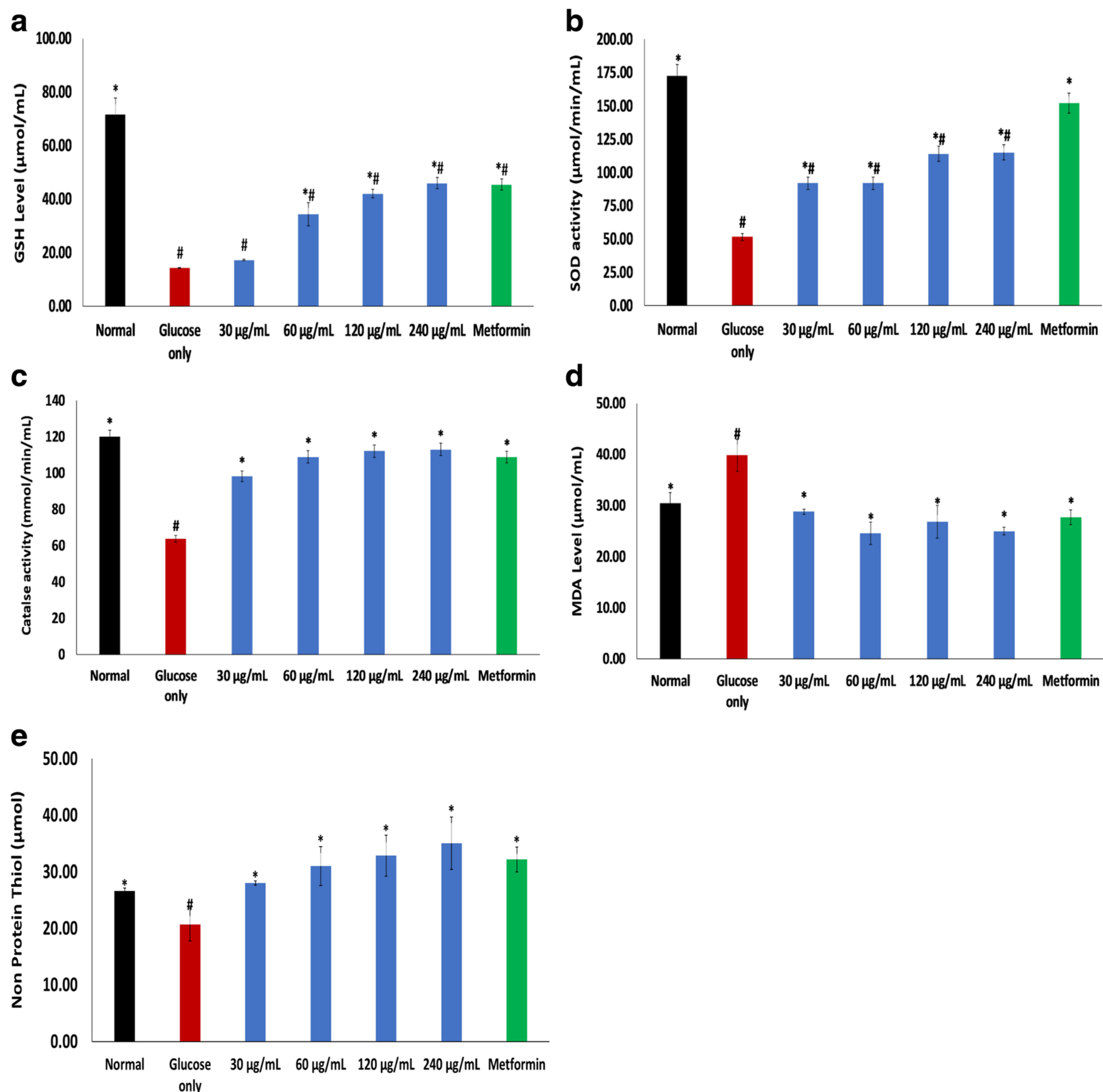

Fig. 2 Effect of *V. amygdalina* infusion on (**a**) GSH level; (**b**) SOD and (**c**) catalase activities; and (**d**) MDA levels in glucose treated brain. Data = mean + SD; n = 3. *Statistically significant compared to Glucose-only treated tissues. Data = mean ± SD; n = 3. *Statistically significant compared to Glucose-only treated tissues; #Statistically significant compared to normal tissues. **e** Effect of *V. amygdalina* infusion on non-thiol proteins levels in glucose treated brain. Data = mean ± SD; n = 3. *Statistically significant compared to Glucose-only treated tissues; #Statistically significant compared to normal tissues

5%CO_2, 95% oxygen and 37 °C conditions for 2 h (Chukwuma and Islam 2015). An incubation without the infusion, served as a control. Metformin (Pharmed Ltd., Durban, South Africa) was used as the standard antidiabetic drug.

At the end of incubation, the brain tissues were removed and homogenized in cold phosphate buffer (50 mM, pH 7.5) with triton X-100. The homogenized tissues were centrifuged at 15,000 rpm for 10 mins at 4 °C, and supernatants stored at −20 °C until further analysis.

All chemicals used were purchased from Sigma-Aldrich, South Africa unless otherwise indicated in manuscript.

Determination of glucose uptake

Glucose concentrations of the buffer were measured before and after the incubation with an automated chemistry analyzer (Labmax Plenno, Labtest Inc., Lagoa Santa, Brazil). Glucose uptake was then calculated using following formula:

$$Glucose\ uptake = \frac{GC1 - GC2}{Weight\ of\ muscle\ tissue\ (g)}$$

Where GC1 and GC2 are glucose concentrations (mg/dL) before and after incubation, respectively.

Determination of oxidative stress and proinflammation parameters

The homgenized tissues were assayed for their reduced glutathione (GSH) (Ellman 1959), Nitric Oxide (NO) (Tsikas 2005), non-protein thiol (Adefegha et al. 2017; Habig et al. 1974) malondialdehyde (MDA) (Chowdhury and Soulsby 2002) levels, catalase (Chance and Maehly 1955), myeloperoxidase (Granell et al. 2003), superoxide dismutase (SOD) (Kakkar et al. 1984) and ATPase (Adewoye et al. 2000) activities.

Molecular docking

Molecular docking was carried out to determine the binding potential of D-glucose to ATPase, catalase and myeloperoxidase. 3D crystal structures of ATPase, catalase, and myeloperoxidase with PDB access code: 4HYT (Laursen et al. 2013), 1TBU (Yang et al. 2013), and 1DNW (Blair-Johnson et al. 2001) respectively were retrieved from protein data bank. The protein resolutions were 3.4 Å, 2.2 Å, and 1.9 Å respectively. *AutoDock tools (Sanner 1999) 1.5.4 was used to determine the suitable grid box size for the potential binding site. The structure of D-glucose was retrieved from PUBMED and optimized using Gaussian 09,(Frisch et al. 2009) This was done to obtain minimized* confromation. The determined dimension was X = 40 Y = 40 Z = 40 with 1.00 Å as the grid spacing. Lamarckian genetic algorithm method was applied to obtain optimum binding site for the ligand. (Yang et al. 2013) Gasteiger charges were computed using the AutoDock Tools graphical user interface supplied by MGL Tools (Morris et al. 2009).

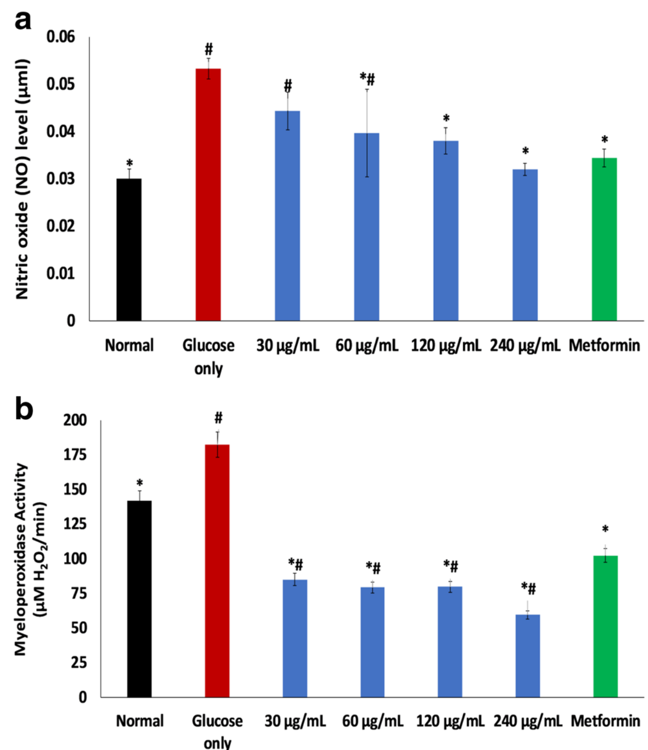

Fig. 3 Effect of *V. amygdalina* infusion on (**a**) NO level and (**b**) myeloperoxidase activity in glucose treated brain. Data = mean ± SD; n = 3. *Statistically significant compared to Glucose-only treated tissues; #Statistically significant compared to normal tissues

Metabolic profiling

Metabolites were extracted from the tissue homogenates using the method as described previously (Chan et al. 2013) with slight modifications (Erukainure et al. 2017b).

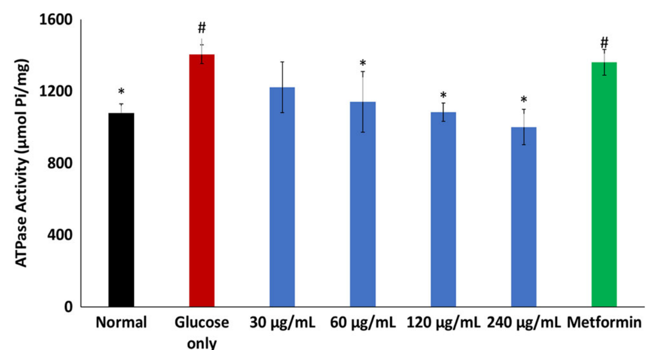

Fig. 4 Inhibitory activities of *V. amygdalina* infusion on ATPase activity in glucose – treated brain. Data = mean ± SD; n = 3. *Statistically significant compared to Glucose-only treated tissues; #Statistically significant compared to normal tissues

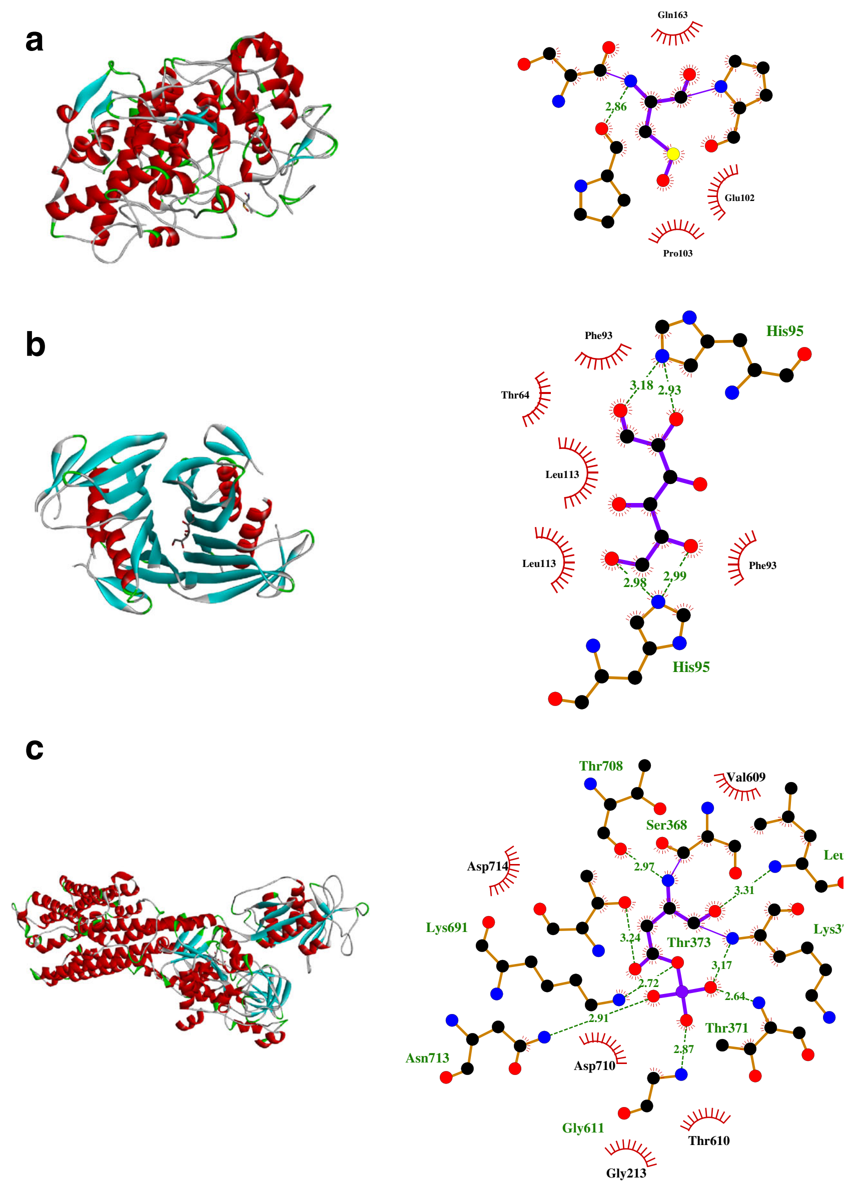

Fig. 5 Molecular interactions of D-glucose with (**a**) myeloperoxidase; (**b**) catalase; and (C) ATPase activities

The extracted metabolites were scanned on Fourier-transform infrared (FT-IR) spectrophotometer (Perkin Elmer Spectrum 400) at room temperature (25–28 °C) at 300–4000 cm^{-1} spectral range. The chemical functional groups were determined by comparing the peak heights and shifts to the IR spectroscopy correlation table.

The metabolic constituents of were determined by subjecting the extracted metabolites to mass spectrometry by Liquid Chromatography – Mass Spectroscopy (LC-MS; Shimadzu LCMS-2020). Direct search of mass spectral data against the Human Metabolome Database (HMDB) was used in identifying metabolites (Wishart et al. 2012).

MetaboAnalyst 4.0 was used in analyzing pathways of significantly altered brain metabolites as described pereviously (Xia and Wishart 2016).

Table 1 Calculated binding energies, core amino acid residue and hydrogen bond distance between residues and ligand from molecular docking

Enzymes	Binding energies (kcal/mol)	Residues	Atoms of the residues	Distance (Å)
ATPase	−5.45	THRE, ASN, LEU, GLY	O, N, H	2.56
Catalase	−4.20	HIS, PHE	H, N	2.06
myeloperoxidase	1.49	PRO, GLN	C, N	2.86

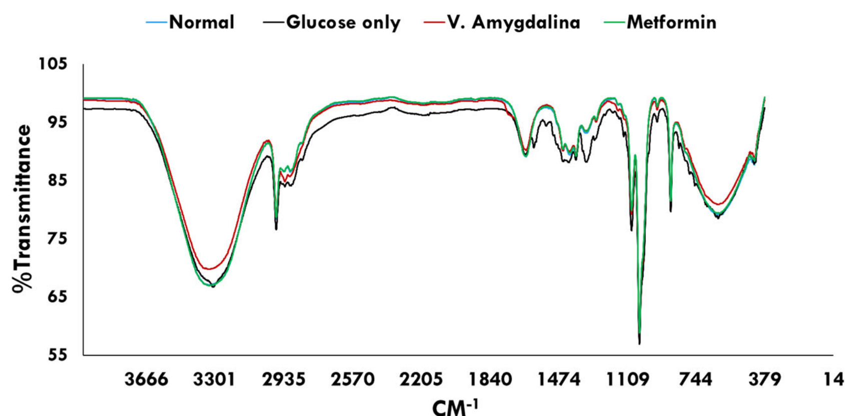

Fig. 6 FTIR Spectroscopy of the effect of *V. amygdalina* infusion on glucose – oxidative brain metabolites

Determination of the phytochemical constituents of *V. amygdalina* hot infusion

The phytochemical constituents of the infused extract of *V. amygdalina* were determined via LC-MS analysis as described by Erukainure et al. (2017b). The compounds were identified using the NIST library online.

In silico prediction of BBB permeability and oral lethal dose toxicity

In silico prediction of BBB permeability and oral lethal dose toxicity of the LC-MS identified compounds of *V. amygdalina* in comparison with their qualitative structure activity relationship (QSAR) and virtual molecular structure activity relationship studies (SARs) were determined using pkCSM – pharmacokinetics (Pires et al. 2015) and PROTOX (Drwal et al. 2014) servers.

Statistics

Statistical significance was determined using one-way analysis of variance (ANOVA), with results presented as mean ± SD. Significant difference was established at $p < 0.05$. Statistical analyses were carried out using IBM SPSS for Windows, version 23.0 (SPSS Inc., Chicago, IL).

Results

Incubation of brain tissues in *V. amygdalina* infusion in the presence of D-glucose, led to significant ($p < 0.05$) increase in

Table 2 Quantitative analysis of FTIR spectra of glucose brain metabolites

Regions (cm^{-1})	Normal (cm^{-1})	Glucose only (cm^{-1})	*V. amygdalina* (cm^{-1})	Metformin (cm^{-1})	Assignment
900–680	–	834.85	–	–	Nitrate
	878.92	879.09	879.09	878.93	1°, 2° amines
1200–900	951.14	951.52	951.34	950.88	Nucleic acid
	1045.07	1045.34	1045.26	1045.15	Nucleic acid
	1086.92	1087.36	1086.99	1086.95	Phosphates
1500–1200	1275.56	1284.50	1275.63	1275.68	Amide 1
	1324.42	1326.02	1331.77	1325.42	Aromatic amines
	1382.87	1382.82	1381.95	1382.53	Phenol –O–H
	1416.85	1420.77	1416.70	1413.51	Nitramines
	1450.26	–	1451.16	1451.38	Amide II
1800–1500	–	1540.09	–	–	Amide II
	–	1606.51	–	–	Amide I
	1648.08	1647.32	1647.63	1643.61	Alkenes
3000–2800	2938.70	2928.95	2975.30	2931.57	Lipid (CH_2)
	2901.91	2896.84	–	2975.91	Lipid (CH_2
	2975.59	2975.52	2975.30	2975.91	Lipid (CH_2)
3180–3030	3327.11	3311.62	3333.92	3331.03	1°, 2° amines, amides

glucose uptake compared to the control (glucose only) and metformin as depicted in Fig. 1. The uptake was dose dependent, with the highest concentration showed the highest uptake. Metformin showed little or no significant effect on glucose uptake.

Incubation of brain tissues with D-glucose significantly ($p < 0.05$) depleted GSH and NTP level, SOD and catalase activities, with concomitant increased levels of MDA as depicted in Fig. 2a–e. These were significantly ($p < 0.05$) reversed on incubation with *V. amygdalina* and metformin respectively, depicting an antioxidative effect.

There was an increased NO level and myeloperoxidase activity on incubation of brain tissues with D-glucose only as shown in Fig. 3a, b, portraying an occurrence of proinflammation. Incubation with the infusion and metformin led to significant ($p < 0.05$) depleted level and activity.

Incubation of brain tissues with D-glucose only, caused a significant ($p < 0.05$) increase in ATPase activity as depicted in Fig. 4. This was significantly reduced dose – dependently on incubation with the infusion, with metformin showing little or no effect.

Molecular docking of d-glucose with catalase, myeloperoxidase, and ATPase revealed significant interactions, with ATPase showing the highest interaction (−5.45 kcal/mol) as depicted in Fig. 5a–c and Table 1. The core amino acid residues and hydrogen bond distance between the residues and ligand are shown in Table 1, with ATPase interaction having the highest number of residues (THRE, ASN, LEU and GLY) and atoms of residues (O, N and H).

Incubation of brain tissues with glucose only, led to the development of nitrate, amide II and amide I functional groups as shown in Fig. 6 and Table 2. Incubation with the infusion and metformin led to removal of these functional groups.

LC-MS characterization of *V. amygdalina* infusion revealed an alkaloid rich extract consisting of nicotinic acid, cumidine, and isoquinoline, 3-methyl- (Fig. 7). Amino acids consisting of l-serine, l-cysteine and l-proline were also identified. Other compounds identified were salicylic acid and γ-octalactone.

Analysis of the metabolites of the normal brain tissues revealed the presence of ganglioside, glucose, cardiolipin, triglyceride, inosine, molybdenum cofactor, monosaccharide, nucleotide, and iduronic acid metabolic intermediates as shown in Table 3. Incubation with d-glucose led to depletion of dTDP-D-glucose and ganglioside GM2 (d18:0/22:1(13Z)), with concomitant generation of 2-keto-glutaramic acid, cysteinyl-tyrosine, acetyl adenylate, CL(18:1(11Z)/18:1(11Z)/18:1(11Z)/18:1(11Z)), Ganglioside GM1 (d18:1/25:0), 4-Methylnonacosane, Ganglioside GT3 (d18:1/16:0) and Ganglioside GT3 (d18:0/16:0). Treatment with the infusion led regeneration of dTDP-D-glucose, with concomitant depletion of P1,P4-Bis(5′-uridyl) tetraphosphate, CL(18:2(9Z,12Z)/18:2(9Z,12Z)/18:2(9Z,12Z)/16:1(9Z)), 2-keto-glutaramic acid and cysteinyl-tyrosine. It also led to the generation of S-formylglutathione and adenosine tetraphosphate. Metformin led to regeneration of the glucose-induced depleted metabolites, with concomitant depletion of cysteinyl-tyrosine, acetyl adenylate, CL(18:1(11Z)/18:1(11Z)/18:1(11Z)/18:1(11Z)), ganglioside GT3 (d18:1/16:0) and ganglioside GT3 (d18:0/16:0), and generation of superoxide, glycerol 3-phosphate and guanosine tetraphosphate adenosine.

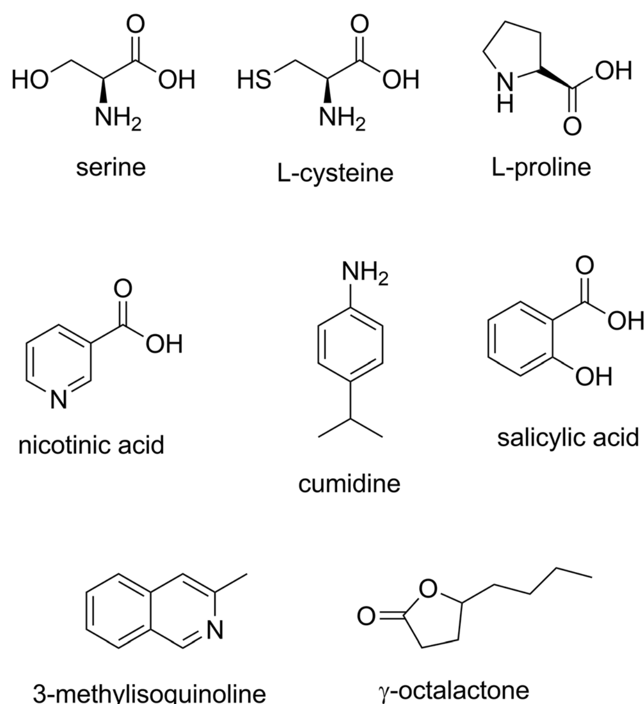

Fig. 7 LC-MS identified compounds of *V. amygdalina* infusion

Pathway enrichment analysis of the identified metabolites revealed metabolic pathways for lactose synthesis, androstenedione, estrone, sucrose, amino sugar, androgen and estrogen, inositol, galactose, porphyrin, sphingolipid, and pyrimidine metabolisms in normal brain tissues (control) as shown in Table 4. These pathways were unaltered on incubation with d-glucose, with activation of pyruvate metabolism pathway. Although incubation with *V. amygdalina* infusion did not affect these pathways, the intensity fold of the pyruvate metabolism pathway was significantly lower than that of glucose-only treated tissues. Treatment with metformin deactivated the pyruvate metabolism pathway, with concomitant activation of de novo triacylglycerol biosynthesis, cardiolipin biosynthesis, degradation of superoxides, glycerol phosphate shuttle, mitochondrial electron transport chain, glycerolipid metabolism, and phospholipid biosynthesis pathways.

Except the amino acids, the other identified compounds of *V. amygdalina* infusion were predicted to be able to cross the blood brain barrier (Table 5). All the identified compounds

Table 3 Identified metabolites in glucose treated brain tissues

Metabolites	Normal Tissues	Glucose only Tissues	*V. amygdalina* Treated Tissues	Metformin Treated Tissues
Uridine 5′-diphosphate	X	X	X	X
Ganglioside GM2	X	X	X	X
1-Phosphatidyl-1D-myo-inositol 3-phosphate	X	X	X	X
Ganglioside GD3 (d18:0/26:0)	X	X	X	X
TG(24:1(15Z)/o-18:0/18:4(6Z,9Z,12Z,15Z))	X	X	X	X
3-Methylellagic acid 8-(4-acetylrhamnoside)	X	X	X	X
UDP-L-iduronate	X	X	X	X
UDP-4-keto-6-deoxy-D-glucose	X	X	X	X
Ganglioside GT3 (d18:1/26:0)	X	X	X	X
dTDP-D-glucose	X	–	X	X
Cyanidin 3-(6″-dioxalylglucoside)	X	X	X	X
Adenosine tetraphosphate	X	X	X	X
ADP-ribose 1″-2″ cyclic phosphate	X	X	X	X
CL(16:0/18:0/16:0/18:1(9Z))	X	X	X	X
Ganglioside GT1b (d18:0/14:0)	X	X	X	X
Ganglioside GM2 (d18:0/22:1(13Z))	X	–	–	X
CL(16:1(9Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z)/22:5(7Z,10Z,13Z,16Z,19Z)/16:1(9Z))	X	X	X	X
Sedoheptulose 1,7-bisphosphate	X	X	X	X
P1,P4-Bis(5′-uridyl) tetraphosphate	X	X	–	X
CL(18:2(9Z,12Z)/18:2(9Z,12Z)/18:2(9Z,12Z)/16:1(9Z))	X	X	–	X
Molybdopterin-AMP	X	X	X	X
Ganglioside GM1 (18:1/22:0)	X	X	X	X
2-Keto-glutaramic acid	–	X	–	X
Cysteinyl-Tyrosine	–	X	–	–
Acetyl adenylate	–	X	X	–
CL(18:1(11Z)/18:1(11Z)/18:1(11Z)/18:1(11Z))	–	X	X	–
Ganglioside GM1 (d18:1/25:0)	–	X	X	X
4-Methylnonacosane	–	X	X	–
Ganglioside GT3 (d18:1/16:0)	–	X	X	–
Ganglioside GT3 (d18:0/16:0)	–	X	X	X
S-Formylglutathione	–	–	X	–
Adenosine tetraphosphate	–	–	X	–
Superoxide	–	–	–	X
Glycerol 3-phosphate	–	–	–	X
Guanosine tetraphosphate adenosine	–	–	–	X

were predicted to be orally safe as they fell between classes 4–6, except for l-serine which fell on class 2.

Discussion

Diminished brain glucose uptake has been recognized as one of the complication of T2D despite of chronic hyperglycemia (Vagelatos and Eslick 2013; Whitlow et al. 2015). This has been attributed to the down regulation of glucose transporters at the BBB, thus reducing facilitative transportation of glucose to the brain (Gejl et al. 2017; Hwang et al. 2017). *Vernonia amygdalina* amongst other medicinal plants have been reported for its antidiabetic and neuroprotective properties. To the best of our knowledge, for the first time, this study reports the ability of *V. amygdalina* to stimulate brain glucose uptake and its effect on brain metabolic pathways and metabolites.

The increased brain glucose uptake by the infusion (Fig. 1) indicates a glucose uptake facilitative potential of *V. amygdalina*. This can be attributed to the identified alkaloid, polyphenol and lactone constituents of the infusion (Fig. 7), as they were predicted to be BBB permeable

(Table 5). Their predicted permeability across BBB may facilitate the transportation of glucose to the brain by the activation of glucose transporters notably GLUTs 1, 3 and 5 which are highly concentrated at the BBB (Simpson et al. 1994). Studies have reported the influence of phytochemicals from medicinal plants, particularly polyphenols, on the activation of glucose transporters (León et al. 2017; Williamson 2013). The reduced glucose uptake in brain tissues incubated with metformin can be attributed to the fact that metformin exhibits its action by the activation of GLUTs 2 and 4, which are less expressed at the BBB (Kellett and Brot-Laroche 2005; Rice et al. 2011). The increased glucose uptake by *V. amygdalina* can also be attributed to its ability to decrease ATPase activities in brain tissues (Fig. 4), as decreased ATPase activity, particularly the Na^+/K^+ ATPase have been implicated in facilitating the glucose transportation across the BBB (Falkowska et al. 2015; Magistretti and Allaman 2015). The high binding energy on docking D-glucose with ATPase (Fig. 5c and Table 1) portrays a strong molecular interaction, which is evident by the increased ATPase activity (Fig. 4) and decreased glucose uptake (Fig. 1) in brain tissues incubated with glucose only. Similarly, the increased ATPase activity in the metformin treated brain tissues (Fig. 4) corroborates its lower glucose uptake (Fig. 1).

Oxidative stress and inflammation have been reported for its influential role in the pathogenesis and progression of neuropathology (Das et al. 2009; Patel 2016), which have been attributed to the high consumption of O_2 and glucose dependence by the brain (Patel 2016). Although the brain's anatomy allows for a reductive environment which minimizes ROS generation, its low endogenous antioxidant system, redox-active metal load, polyunsaturated fatty acids, and excitotoxic and auto-oxidizable neurotransmitters dependence makes it prone to oxidative stress (Butterfield et al. 2001; Huang et al. 2004; Patel 2016). The depleted levels of GSH, NPT, and increased SOD and catalase activities, with concomitantly increased MDA level in brain tissues incubated with glucose only (Fig. 2a–e) depicts an occurrence of oxidative stress. This can be attributed to the activation of pyruvate metabolism pathway (Table 4). Activation of this pathway will lead to the production of lactate with concomitant generation of essential cofactor, NAD^+. The continuous drive of this pathway lead to accumulation of lactate and increased generation of NAD^+ which sustains the glycolytic flux, which in turn increases the turnover of glycolytic production of electron donor, NADH. This electron donor has been implicated in the production of ROS, as it inhibits the electron transport at complex II leading to the reduction of O_2 to $O_2^{\cdot-}$ (Brownlee 2001; Du et al. 2001). The activation of this pathway can also be

Table 4 Identified metabolic pathways in glucose treated brain tissues

Metabolic pathways	Normal tissues	Glucose only tissues	*V. amygdalina* treated tissues	Metformin treated tissues
Lactose synthesis	X	X	X	X
Androstenedione metabolism	X	X	X	X
Estrone metabolism	X	X	X	X
Starch and sucrose metabolism	X	X	X	X
Amino sugar metabolism	X	X	X	X
Androgen and estrogen metabolism	X	X	X	X
Inositol metabolism	X	X	X	X
Galactose metabolism	X	X	X	X
Porphyrin metabolism	X	X	X	X
Sphingolipid metabolism	X	X	X	X
Pyrimidine metabolism	X	X	X	X
Pyruvate metabolism	–	X	X	–
De novo triacylglycerol biosynthesis	–	–	–	X
Cardiolipin biosynthesis	–	–	–	X
Degradation of superoxides	–	–	–	X
Glycerol phosphate shuttle	–	–	–	X
Mitochondrial electron transport chain	–	–	–	X
Glycerolipid metabolism	–	–	–	X
Phospholipid biosynthesis	–	–	–	X

Table 5 BBB Permeability and Predicted toxicity of compounds from V. amygdalina infusion

Compounds	BBB Permeability	Predicted LD50 (mg/kg)	Predicted Toxicity Class
L – Serine	No	2000	2
L – Cysteine	No	660	4
L - Proline	No	2078	5
Nicotinic Acid	Yes	3720	5
Cumidine	Yes	500	4
Salicylic acid	Yes	480	4
Isoquinoline, 3-methyl-	Yes	1230	4
γ-Octalactone	Yes	4390	5

attributed to the decreased glucose uptake, owing to the need for the brain to switch energy source from glucose to ketones. This is evident by the presence of the ketone metabolite, 2-keto-glutaramic acid in the glucose-only treated tissue (Table 3). 2-keto-glutaramic acid can also act as a substrate for the enzyme, alanine transaminase which catalyzes the reversible conversion of alanine to pyruvate. The presence of sphingolipids and triglyceride derivatives (Table 3) in the glucose-only treated brain tissue, may be responsible for the increased MDA level (Fig. 2d) as they can act as substrates for lipid peroxidation. The strong molecular interactions between D-glucose and catalase (Fig. 5b and Table 1) suggests the potential of D-glucose to inhibit catalase activity.

The increased GSH and NTP levels, and SOD and catalase activities, with concomitant depletion of MDA level in the treated brain tissues indicate an antioxidative effect. The antioxidative effect of the infusion can be attributed to its identified phytochemical constituents particularly nicotinic acid, salicylic acid and γ-Octalactone, as these compounds have been reported for their antioxidant and neuroprotective activities (De La Cruz et al. 2004; Shoaib et al. 2017; Tupe et al. 2011). This can also be attributed to their predicted permeability across the BBB (Table 5), which corroborates other reports on the ability of polyphenols and alkaloids to cross the BBB (Youdim et al. 2003; Zhang et al. 2017). The standard antidiabetic drug, metformin caused an inhibition of the pyruvate metabolism, with concomitant activation of the glycerol phosphate shuttle and mitochondrial electron transport chain (Table 4). This indicates the activation of a proper channel of transporting electron donors generated by the glycolytic pathway, thus mopping up free radicals while generating ATPs for the brain use. This is corroborated by the superoxide and glycerol 3-phosphate metabolites (Table 3) and superoxide degradation pathway (Table 4), which is evident by its high SOD activity (Fig. 2b). The activated lipid metabolic pathways in the metformin treated tissues, indicates a maintenance of tissue integrity which may also be responsible for the decreased MDA level (Fig. 2d).

The increased NO level and myeloperoxidase activity (Fig. 3a, b) indicates an occurrence of proinflammation in the brain tissue incubated in glucose only. This is evident by the presence of nitrate functional group (Fig. 6 and Table 2). Similarly, the molecular interaction between D-glucose and myeloperoxidase (Fig. 5a and Table 1) indicates the potential of the former to activate the latter. The reversed level and activity on incubation with *V. amygdalina* infusion and metformin indicates an anti-proinflammatory activity, which is evident by the absence of the nitrate functional groups (Fig. 5a and Table 1). This corroborates previous reports on the anti-proinflammatory activity of *V. amygdalina* (Farombi and Owoeye 2011; Georgewill and Georgewill 2010), and can be attributed to the identified phytochemical constituents (Fig. 7) as well as their predicted BBB permeability (Table 5). Thus, further indicating the neuroprotective effect of *V. amygdalina*.

The predicted toxicity of the identified phytochemical constituents (Table 5) may indicate a relative safety of the infusion when ingested orally.

Conclusion

These results of this study suggest the ability of *V. amygdalina* to stimulate glucose uptake in brain tissue, with concomitant antioxidative and anti-proinflammatory activities. Thus, indicating its neuroprotective potential against diabetic brain. This can be attributed to the identified phytochemicals and their permeability across the BBB. Thus, further giving credence to the reports and folkloric use of this plant in the treatment of neurodegenerative diseases.

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Compliance with ethical standards

Conflict of interest The authors report no conflict of interest.

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