Obade et al. Clinical Phytoscience (2018) 4:9
https://doi.org/10.1186/s40816-018-0068-9

Clinical Phytoscience

ORIGINAL CONTRIBUTION Open Access

Neuromodulatory effect of solvent fractions of Africa eggplant (Solanium dadyphyllum) against KCN-induced mitochondria damage, viz. NADH-succinate dehydrogenase, NADH- cytochrome c reductase, and succinate-cytochrome c reductase

Efe Obade[1], Omotayo B. Ilesanmi[1,2*], Olamide Crown[1], Afolabi C. Akinmoladun[1], Tolulope M. Olaleye[1] and Akintunde A. Akindahunsi[1]

Abstract

Background: In the past few years, there has been a tremendous increase in the number of plant-based health supplements with respect to their safety and efficacy in diseases treatment and prevention. *Solanum dasyphyllum*, also known as Africa eggplant is ethnomedicinally used as an antivenom, pain reliever and anticonvulsant in various part of Nigeria, however, there is no scientific data to support some of these claims.

Methods: This study evaluated the protective effect of solvent fractions of *Solanum dasyphyllum*, hexane fraction of *S. dasyphyllum* (HFSD), dichloromethane fraction of *S. dasyphyllum* (DFSD), ethylacetate fraction of *S. dasyphyllum* (EAFSD), methanolic fraction of *S. dasyphyllum* (MFSD) and crude fraction of *S. dasyphyllum* (CFSD) on cyanide-induced oxidative stress and neurotoxicity in vitro in the cerebral cortex. Neuroprotective activities were evaluated by assaying for markers of oxidative stress, neurotoxicity and electron transport system enzymes via evaluating lipid peroxidation (LPO), protein carbonyl (PC), reduced glutathione (GSH), acetylcholinesterase (AChE), NADH-succinate dehydrogenase (NSD), NADH-cytochrome c reductase (NCR), and succinate-cytochrome c reductase (SCR) in the homogenate of cerebral cortex.

(Continued on next page)

* Correspondence: ilesanmiob@fuotuoke.edu.ng
[1]Phytomedicine , Biochemical Pharmacology and Toxicology Laboratories, Department of Biochemistry, School of Sciences , The Federal University of Technology Akure, P.M. B. 704 Akure, Onto State, Nigeria
[2]Department of Biochemistry, Faculty of Science, Federal University Otuoke, P.M.B. 126 Yenagoa, Bayelsa State, Nigeria

Springer Open

© The Author(s). 2018 **Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

(Continued from previous page)

Results: The results showed that all solvent fractions of *S. dasyphyllum* significantly ameliorated cyanide-induced oxidative stress ($P < 0.05$). It inhibited the activity of acetylcholinesterase-HFSD (68.42 ± 5.37%), DFSD (36.32 ± 5.45%), EFA (20 ± 0.69%), MFSD (33.16 ± 4.8%) and CFSD (35.79 ± 2.8%), increased the activity of NSD [HFSD (94.74 ± 7.3%), EAFSD (78.95 ± 5.4%) and CFSD (60.53 ± 4.6%)], while DFSD (− 5.26 ± 1.4%) and MFSD (− 7.9 ± 0.4%) had a negative effect, increased the activity of NCR [HFSD (91.89 ± 7.1%), DFSD (90.54 ± 8.2%), EAFSD (62.16 ± 4.7%); MFSD (306.76 ± 7.2%) and CFSD (154.0 ± 8.1%)]. All the solvent fractions also significantly increased the activity of SCR [HFSD (70.59 ± 3.8%), EAFSD (58.82 ± 6.4%), MFSD (88.24 ± 9.0%) CFSD (76.47 ± 8.2%)] apart from DFSD (− 5.88 ± 1.2%) in rat cerebral cortex.

Conclusion: This result shows that *S. dasyphyllum* has neuroprotective activities, however HFSD shows the most potent bioactivities in maintaining mitochondria integrity by preserving the electron transport system. Further work can be done on isolating and characterizing the bioactive compound in HFSD for novel natural product in the treatment of neurological disorders.

Keywords: Mitochondria, Electron transport enzymes, Alternative medicine, *Solanum dasyphyllum*, Oxidative stress, Neurological disorders

Background

The brain is one of the complex and delicate organ in the body, such that some of the cells survive the entire lifespan of human [1]. The brain is a key component of the nervous system, thus, it affects various neuromuscular activities in the body. The aging of brain cells causes an increase in reactive oxygen species generation, coupled with low level of antioxidant system in the brain leads to oxidative stress (OS) [2]. Thus several normal physiological functions such as memory and voluntary movement controlled sby cortex are reduced or lost, while postural stability, movement coordination coordinated by the cerebellum are also lost. Deficient or loss of this function is termed neurodegenerative diseases [3]. With increase in aged population, it is estimated that by 2050, the number of patient suffering from neurodegenerative diseases will double [4].

Mitochondria are described as “the powerhouse of the cell” because they generate most of the cell’s supply of adenosine triphosphate (ATP), used as a source of chemical energy. In addition to supplying cellular energy, mitochondria are involved in other tasks, such as signallingcellular differentiation, and cell death, as well as maintaining control of the cell cycle and cell growth. Mitochondria have been implicated in several human diseases, including mitochondrial disorders, cardiac dysfunction, heart failure and neurological disorders [4, 5].

Cyanide is often found joined with other chemicals to form compounds. Examples of simple cyanide compounds are hydrogen cyanide sodium cyanide and potassium cyanide and hydrogen cyanide is the main form present in the air. Cyanide can be released into the environment during the course of industrial usage, from smoke or vehicles exhaust containing the incomplete combustion products of nitrogen-containing organic polymers. Also, several plants contain cyanogen glycosides that can release hydrogen cyanide upon biodegradation or digestion [6].

Cyanides are highly toxic due to their binding to the ferric form of vital cellular enzymes such as cytochrome oxidase, succinic dehydrogenase and superoxide dismutase, with particular interest on the electron transport system in the mitochondria [7]. The binding of cyanide to this mitochondrial enzyme prevent the transport of electron from cytochrome c to oxygen resulting in disruption of the electron transport chain and inability of cells to aerobically produce ATP. This leads to hypoxia and tissue damage, with the most vulnerable tissues being those with high oxygen demands such as brain and heart [8, 9]. Other toxic effect of cyanide includes binding to affecting neurotransmitter receptor N-methyl-daspartate (NMDA) receptor to increase the release of glutamate and elevate the activity of acetylcholinesterase, an enzyme that catalyse the degradation of acetylcholine and also increase the generation of reactive oxygen species [10]. Although antidotes to cyanide poisoning exist, these have limitations and side effects [11]. Therefore, search for highly effective and safe agents across different scenarios of cyanide toxicity is essential.

Some Herbal products that are perceived to be more effective and have fewer side effects in therapeutics are gaining increasing prominence than synthetically derived drugs in healthcare [12]. *Solanum dasyphyllum*, otherwise known as the African eggplant or gboma leaves has been used locally as antidotes (venomous stings, bites etc),treatment of tooth aches, stomach pains, parasitic infections, swellings etc. It is known to have anticonvulsant, neuromodulatory and cardiovascular (hypotensive) properties.

The present study was designed to ascertain the potential of *S. dasyphyllum*, a locally used anticonvulsant plant, to protect against mitochondria damage and neurotoxicity induced by KCN .

Methods

Chemicals and reagents

Thiobarbituric acid (TBA), trichloroacetic acid (TCA), ubiquinone, perchloric acid (PCA), benzylamine hydrochloride (BAHC), sodium dodecyl sulphate (SDS), ethylene diamine tetraacetic acid (EDTA), acetylcholine iodide, 2,4-dinitrophenyl hydrazine, cytochrome and reduced nicotinamide adenine dinucleotide phosphate (NADH) were obtained from Sigma-Aldrich (St-Louis, MO, USA). Randox Kits were used for total protein assay purchased from Randox laboratory lid, United Kingdom. All other chemicals and reagents were of analytical grade.

Plant collection and preparation of extract

Experimental animals

Male Wister rats with weighing 200 g were bred and housed at the animal house of the Department of Animal Production and Health, Federal University of Technology, Akure, Nigeria. Animals were kept under standard laboratory condition and fed with laboratory chow (vital feed lagos, Nigeria) and water ad libitum. All animals' experimental protocols conformed to the guidelines of National Institute of Health for laboratory animals.

Identification and collection of plant materials.

African Egg Plant (*Solanum dasyphyllum)* belonging to the family Solanaceae. Leaves of the plant was collected, identified at obafemi awolowo university ile-ife, with the voucher number IFE-17489 and the leaves were air dried in the laboratory (25–28 °C) and grounded into powder form with the aid of an electric blender.

Sample preparation

The powdered forms of the leaf were soaked with absolute methanol for 72 h with constant stirring and was filtered using Whatman No1 filter paper, and the filtrate was concentrated using a rotary evaporator, followed by lyophilizing to dryness.

Solvent partitioning

The crude extract of *S. dasyphyllum* was subjected to solvent partitioning using n-hexane, dichloromethane ethylacetate and methanol (according to their polarity). 15 g of the crude extract was weighed, dissolved in 100 ml of warm water and poured into inclined separating funnel. To obtain hexane fraction of *S. dasyphyllum* (HFSD), 600mls of n-hexane was added into the separating funnel, shake gently and allowed to separate on standing. The residue and the supernatant fractions were collected. The residue was returned back into the separating funnel and 800mls of n-hexane was added, shaken, and allowed to separate on standing. The same procedure as above was followed for the collection of dichloromethane (DCM) fraction of *S. dasyphyllum* (DFSD) and 800mls of DCM was used, ethylacetate (EA) fraction of *S. dasyphyllum* (EAFSD), 700mls of EA was used and methanolic fraction of *S. dasyphyllum* (MFSD) 1000mls of methanol was used. The fractions (n- hexane, dichloromethane, ethylacetate and methanol) were concentrated to dryness using rotary evaporator and weighed.

Cerebellum and cortex preparation

Cortex and cerebellum preparation was done by differential centrifugation method [13]. The rats were sacrificed by cervical dislocation without anaesthesia and the brain was rapidly excised on the petri dish placed on ice, the blood and eternal vessels was carefully removed. The cerebellum and the cerebral cortex regions of the brain were carefully dissected, weighed and separately homogenized in 0.1 M phosphate buffer at pH 7.4 with a manual glass-teflon homogenizer on ice to obtain 10% *w*/*v* homogenate and centrifuged at 6000 rpm at 4 °C for 25 min to obtain the supernatant, stored in an amber bottle and refrigerated, which was used for biochemical analyses.

Evaluation of the neuroprotective effect of S. dasyphyllum in rat brain homogenate challenged with cyanide

Experimental design To investigate the neuroprotective activities of *S. dasyphyllum*, the cerebellum and cortex homogenate mixture were incubated with 0.09 mM of potassium cyanide (KCN), in the presence or absence of varying concentrations of crude extracts and fractions (HFSD, DFSD, EAFSD and MFSD) of *S. dasyphyllum* leaves at 37 °C for 1 h respectively was used to induce mitochondria damage under in vitro condition.

Group I: **Control;** contained homogenate and distilled water.
Group II: **KCN;** contained homogenate and toxicant.
Group III: **5** μg/**ml + KCN;** contained homogenate and (5 μg) extract and toxicant.
Group IV: **10 μg/ml + KCN;** contained homogenate and (10 μg) extract and toxicant.
Group V: **20 μg/ml + KCN;** contained homogenate and (20 μg) extract and toxicant.
Group VI: **30 μg/ml + KCN;** contained homogenate and (30 μg) extract and toxicant.
Group VII: **40 μg/ml + KCN;** contained homogenate and (40 μg) extract and toxicant.
Group VIII: **50 μg/ml + KCN;** contained homogenate and (50 μg) extract and toxicant.

Determination of reduced glutathione (GSH) concentration

Estimation of GSH was determined according to the method of [14]. Sample (100 μl) was added to 900 μl of distilled water and 1500 μl of the precipitating solution

(Sulphosalicyclic acid) was mixed with the sample. The rate of addition was not critical. A blank was prepared by replacing the volume of sample with distilled water i.e. a total of 1000 μl of distilled water. The mixture was then allowed to stand for approximately 5 min and then filtered. At the end of the fifth minute, 500 μl of filtrate was added to 250 μl of 0.1 M phosphate buffer. Finally 2250 μl of the Ellman's reagent was added. The optical density was measured at 412 nm and the amount of reduced GSH was determined using an extinction coefficient of 9600 $M^{-1}CM^{-1}$.

Evaluation of lipid peroxidation (LPO)

The LPO inhibitory activity was evaluated by measuring the formation of TBA reactive substances (TBARS) according to the method of [15]. An aliquot of 0.4 ml of the sample was mixed with 1.6 ml of TRIS-KCl buffer to which 0.5 ml of 30% TCA was added. Then 0.5 ml of 0.75% TBA was added and placed in a water bath for 45 min at 80 C. This was then cooled in ice and centrifuged at 3000 g. The clear supernatant was collected and absorbance measured against a reference blank of distilled water at 532 nm. The TBARS level was calculated according to the method of [16]. LPO in units/mg protein was computed with a molar extinction coefficient of 1.56×10^5 M^{-}

Determination of protein carbonyl (PC) content

PC content in the brain regions was determined according to the method of [17]. Brain supernatant was incubated with DNPH for 60 min at room temperature. Following precipitation by adding 20% TCA, the pellet was washed with acetone and dissolved in 1 ml of TRIS buffer containing sodium dodecyl sulphate (8% *w*/*v*, pH 7.4). The absorbance was measured at 360 nm and expressed as nmol carbonyls/mg protein.

Evaluation of NADH – Cytochrome c and succinate – cytochrome c reductase activity [18]

Procedure The activities of NADH–cytochrome C reductase (NCR) and succinate–cytochrome C reductase (SCR) were measured in brain mitochondria as described previously [18]. Briefly mitochondrial protein (0.1 mg) was added to phosphate buffer (0.1 M, pH 7.4) containing NADH (0.2 mM) and KCN (1 mM). The reaction was initiated by the addition of 0.1 mM cytochrome C giving a final volume of 1 ml reaction mixture and decrease in absorbance was monitored for 3 min at 550 nm. The activity was expressed as nmol cytochrome C reduced/min/mg protein 273 (€-19.6/mM/cm). To determine the activity of SCR, the substrate succinate (20 mM) was used.

Evaluation of NADH – Succinate dehydrogenase (NSD) activity [19]

Briefly, 0.05 ml of the reaction mixture was incubated with 0.8 ml phosphate buffer (50 mM), 0.1 ml sodium succinate (0.01 M) and 0.025 ml p-iodonitro tetrazolium (INT) (2.5 μg/ml) at room temperature for 10 min. After incubation, 0.5 ml TCA (10%) and 0.5 ml ethylacetate/ethanol/TCA (5:5:1, v: v: w) was added. Absorbance was read at 490 nm. Results expressed as nmol of INT/ mg protein.

Evaluation of Acetylcholineterase (AChE) activity

AChE activity was evaluated according to [20]. DTNB (0.01 M, 0.1 ml) was added to 2.6 ml of 0.1 M phosphate buffer (pH 8.0). Sample (0.04 ml) was added to the mixture followed by incubation for 5 min. Then, 0.04 ml of substrate (0.075 M acetylthiocholine iodide) was added to the reaction mixture. The readings were taken at 420 nm continuously for 3 min at 30 s intervals. AChE activity was calculated using a molar extinction coefficient 1.36 × 10 – 4 M – 1 cm – 1 and expressed in μmol–1 min – 1 mg protein– 1.

Evaluation of Total protein concentration

Total protein level This was determined using an assay kit from Randox Laboratory Ltd., Antrim, UK following the instructions of the manufacturer.

Statistical analysis

Results were expressed as mean ± standard deviation (SD). Statistical differences between means were determined by oneway analysis of variance (ANOVA) followed by Duncan's test. $P < 0.05$ was considered statistically significant.

Results

Table 1 reveals that the level of AchE was significantly higher in KCN intoxicated untreated group when compared with control group ($P < 0.05$), while in the *Solanum dasyphyllum* treated group, the activity significantly decreased as compared to the induced group (P < 0.05) in a non concentration-dependent manner. It can also be observed from the results that EAFSD at lower concentrations possess a better anticholinesterase activity as compared to other fractions.

Tables 2, 3 and 4 reveals the protective effect of *S. dasphyllum* against cyanide inhibitory action against the activity of electron transport chain enzymes (NCR, SCR and) in the cerebrocrotex. The figures showed that the protective activity of each fractions varies with the enzyme assayed for. While DFSD was generally protective against the inhibitory effect of KCN on NCR. MFSD was protective in all except NSD where there was no significant difference between the enzyme activity in the

Table 1 Anticholinesterase activity of solvent fractions of *S. dasyphyllum* in rats cerebrocortex exposed to potassium cyanide

	Acetylcholilnesterase activity (umole of acetylcholine hydrolyzed/min/mg protein)				
Concentration (μg/ml)	HFSD	DFSD	EAFSD	MFSD	CFSD
Control	0.44 ± 0.045	0.44 ± 0.045	0.44 ± 0.045	0.44 ± 0.045	0.44 ± 0.045
KCN	0.95 ± 0.06	0.95 ± 0.06	0.95 ± 0.06	0.95 ± 0.06	0.95 ± 0.06
5	0.61 ± 0.08(35.79%)	0.605 ± 0.05(36.32%)	0.76 ± 0.10(20%)	0.635 ± 0.9(33.16%)	0.61 ± 0.08(35.79%)
10	0.59 ± 0.06(37.89%)	0.605 ± 0.06(36.32%)	0.925 ± 0.09(2.63%)	0.6 ± 0.08(36.84%)	0.6 ± 0.07(36.84%)
20	0.63 ± 0.1(30.52%)	0.605 ± 0.08(36.32%)	1.06 ± 0.14(−11.58%)	0.595 ± 0.087(37.37%)	0.59 ± 0.1(37.89%)
30	0.66 ± 0.1(30.53%)	0.605 ± 0.08(36.32%)	0.89 ± 0.09(6.32%)	0.59 ± 0.08(37.89%)	0.59 ± 0.07(37.89%)
40	0.65 ± 0.07(31.58%)	0.6 ± 0.09(36.84%)	0.62 ± 0.08(34.74%)	0.595 ± 0.08(37.37%)	0.6 ± 0.09(36.84%)
50	0.59 ± 0.07(37.89%)	0.605 ± 0.08(36.32%)	0.6 ± 0.06(36.84%)	0.635 ± 0.1(33.16%)	0.59 ± 0.09(37.89%)

Results are expressed as means±SD ($n = 3$)
HFSD Hexane fraction of *Solanum dasyphyllum*, *DFSD* Dichloromethane fraction of *Solanum dasyphyllum*, *EAFSD* Ethyl Acetate fraction of *Solanum dasyphyllum*, *MFSD* Methanol fraction of *Solanum dasyphyllum*, *CFSD* Crude fraction of *Solanum dasyphyllum*
* percentage increase in activity as compared to toxicant

fraction and KCN group. HFSD, EFSD and CFSD significantly reverse the inhibitory activity of KCN in all the ETC enzymes evaluated.

Figures 1, 2 and 3 showed the antioxidant effect of all the fractions against KCN induced toxicity in the cerebrocortex. The crude and hexane fractions showed a significant increase in the concentration of GSH as compared to the KCN group, while there was no significant difference between the cyanide group and all other fractions ($P < 0.05$). However, all the fractions significantly prevent PC formation and LPO in the cerebrocortex as compared to the control ($P < 0.05$).

Discussion

Findings in the results showed that *S. dasyphyllum* reverse the neurotoxicity of KSN by preserving mitochondria integrity through the preservation of electron transport chain enzymes. The ease of accessibility and lower toxicity of phytochemicals has rekindled the global research on medicinal plants as a drug of choice in the treatment of diseases. The neuromuscular and hypotensive properties of phytochemicals isolated from *S. dasyphylum* has also been reported [21], this includes, coumarin and scopoletin [21]. Neurodegenerative disease such as Alzheimer's disease and Parkinson's disease has been linked to mitochondria dysfunction [22]. Cyanide is also known to be an inhibitor of complex IV in the electron transport chain, leading to severe depletion of ATP and cell death that is dependent on energy impairment). KCN has been reported to alter the metabolism of acetylcholine via increasing the activity of AChE [10]. The current data corroborated the increased activity of AChE in the cerebrocortex by KCN. KCN has been known to stimulate hypoxia cell death via the reduction in the availability of acetylcholine to the synaptic cells [23]. All the solvent fractions significantly reverse the increased acetylcholinesterase activity induced by KCN. EAFSD was not effective at low concentration as compared to other solvent fractions, however, at

Table 2 Effect of solvent fractions of *S. dasyphyllum* on NADH -cytochrome c reductase activity in rat cerebrocortex exposed to potassium cyanide

	NADH cytochrome c reductase activity (nmol of cytochrome c reduced/min/mg protein)				
Concentration (μg/ml)	HFSD	DFSD	EAFSD	MFSD	CFSD
Control	0.775 ± 0.051	0.775 ± 0.051	0.775 ± 0.051	0.775 ± 0.051	0.775 ± 0.051
KCN	0.027	0.027	0.027	0.027	0.027
5	0.71 ± 0.07(91.89%)*	0.705 ± 0.05(90.54%)	0.6 ± 0.071(62.16%)	1.505 ± 0.06(306.76%)%)	0.94 ± (0.09(154.05%)
10	0.695 ± 0.06(87.84%)	0.98 ± 0.87(164.87%)	0.565 ± 0.04(52.70%)	0.445 ± 0.04(20.27%)	0.745 ± 0.067(101.35%)
20	0.63 ± 0.05(70.27%)	0.85 ± 0.08(129.73%)	0.585 ± 0.07(58.11%)	0.605 ± 0.05(63.5%)	1.115 ± 0.09(201.35%)
30	0.67 ± 0.06(81.08%)	0.865 ± 0.07(133.78%)	0.55 ± 0.05(48.65%)	0.675 ± 0.054(82.43%)	0.695 ± 0.06(87.84%)
40	0.765 ± 0.06(106.76%)	0.745 ± 0.105(101.35%)	0.585 ± 0.05(58.11%)	0.625 ± 0.05(68.92%)	0.57 ± 0.04(54.05%)
50	1.005 ± 0.06(171.62%)	0.85 ± 0.12(129.73%)	0.465 ± 0.04(25.68%)	0.585 ± 0.05(58.11%)	0.705 ± 0.06(90.54%)

Results are expressed as means±SD ($n = 3$)
HFSD Hexane fraction of *Solanum dasyphyllum*, *DFSD* Dichloromethane fraction of *Solanum dasyphyllum*, *EAFSD* Ethyl Acetate fraction of *Solanum dasyphyllum*, *MFSD* Methanol fraction of *Solanum dasyphyllum*, *CFSD* Crude fraction of *Solanum dasyphyllum*
* percentage increase in activity as compared to toxicant

Table 3 Effect of solvent fractions of *S. dasyphyllum* on Succinate- cytochrome c reductase in rat cerebrocortex exposed to potassium cyanide

	Succinate cytochrome c reductase activity (nmol of cytochrome c reduced/min/mg protein)				
Concentration (μg/ml)	HFSD	DFSD	EAFSD	MFSD	CFSD
Control	0.155 ± 0.04	0.155 ± 0.04	0.155 ± 0.04	0.155 ± 0.04	0.155 ± 0.04
KCN	0.085 ± 0.002	0.085 ± 0.002	0.085 ± 0.002	0.085 ± 0.002	0.085 ± 0.002
5	0.145 ± 0.3(70.59%)	0.08 ± 0.017(−5.88%)	0.135 ± 0.019(58.82%)	0.16 ± 0.03(88.24%)	0.15 ± 0.03(76.47%)
10	0.285 ± 0.041(235.29%)	0.07 ± 0.001(−17.65%)	0.21 ± 0.051(147.06%)	0.205 ± 0.207(141.17%)	0.13 ± 0.03(52.94%)
20	0.15 ± 0.041(76.47%)	0.06 ± 0.02(−29.41%)	0.14 ± 0.03(64.71%)	0.145 ± 0.03(70.59%)	0.125 ± 0.042(47.06%)
30	0.14 ± 0.03(64.71%)	0.09 ± 0.01(5.88%)	0.16 ± 0.05(88.24%)	0.22 ± 0.03(158.82%)	0.155 ± 0.021(82.35%)
40	0.165 ± 0.03(94.12%)	0.075 ± 0.006(−11.76%)	0.175 ± 0.03(105.88%)	0.135 ± 0.019(58.82%)	0.13 ± 0.01(52.94%)
50	0.175 ± 0.03(105.88%)	0.085 ± 0.0215(0%)	0.155 ± 0.04(82.35%)	0.135 ± 0.02(58.82%)	0.155 ± 0.02(82.35%)

Results are expressed as means±SD (n = 3)
HFSD Hexane fraction of *Solanum dasyphyllum*, *DFSD* Dichloromethane fraction of *Solanum dasyphyllum*, *EAFSD* Ethyl Acetate fraction of *Solanum dasyphyllum*, *MFSD* Methanol fraction of *Solanum dasyphyllum*, *CFSD* Crude fraction of *Solanum dasyphyllum*
* percentage increase in activity as compared to toxicant

higher concentration, it significantly reversed the activity of AChE. One of the key targets in the development of drugs for the treatment of neurological disorders is the search for AChE inhibitors [10]. The AChE inhibitory activity of all the solvent fraction of *S. dasyphyllum* might be as a result of various phytochemicals present in the plants, such as alkaloids, flavonoids, coumarin, and steroids (unpublished), that have been reported to possess anticholinesterase activity [24]. Thus, *S. dasyphyllum* can be classified among anticholinesterase plants in the treatment of AChE-related neurological disorders.

The activity of ETC is central to the integrity of mitochondria, in the present study, KCN being a high-affinity ETC blocker significantly disrupted the enzyme activities across the mitochondria membrane in the cerebrocortex, with reduced activity of enzymes involved in electron transport (NADH-cytochrome c reductase [NCR], succinate-cytochrome c reductase [SCR] and NADH-succinate dehydrogenase [NSD] as compared to the control in the cerebrocortex. The sensitivity of the enzymes to KCN was clearly observed in the experiment, with NCR more sensitive to KCN than SCR. This inhibition can lead to energy deficit and electron leakage, further increasing free radical generation [24–26]. All the solvent fractions of *S. dasyphyllum* were able to reverse the inhibitory effect of KCN on ETC enzymes. One of the central point in the mechanism of disease prevention by phytochemicals is through the protection of mitochondria against injury [27–29]. Such phytochemicals are found in plants like baicalein, *Parkia biglobosa, Antiaris africana* and curcumin [30–33]. KCN significantly elevated PC and LPO generation in the cerebrocortex. This might be as a result of the inhibitory activity of KCN on the ETC, leading to increased generation of ROS, that reacts with functional protein and lipids, causing loss of function. This is not different from the report of [18]. The low level of LPO and PC observed in the cerebrocortex upon co-treatment with different solvent fractions of *S. dasyphyllum* suggesting a possible antioxidative role of

Table 4 Effect of *S. dasyphyllum* on NADH- succinate dehydrogenase in rat cerebrocortex exposed to potassium cyanide

	NADH succinate dehydrogenase activity (optical density/min/mg protein)				
Concentration (μg/ml)	HFSD	DFSD	EAFSD	MFSD	CFSD
Control	0.395 ± 0.027	0.395 ± 0.027	0.395 ± 0.027	0.395 ± 0.027	0.395 ± 0.027
KCN	0.19 ± 0.01	0.19 ± 0.01	0.19 ± 0.01	0.19 ± 0.01	0.19 ± 0.01
5	0.37 ± 0.044(94.74%)	0.18 ± 0.036(−5.26%)	0.34 ± 0.034(78.95%)	0.175 ± 0.038(−7.9%)	0.305 ± 0.08(60.52%)
10	0.475 ± 0.048(150%)	0.175 ± 0.037(− 7.89%)	0.38 ± 0.04(100%)	0.18 ± 0.026(−5.26%)	0.305 ± 0.08(60.53%)
20	0.41 ± 0.0615(115.79%)	0.18 ± 0.046(−5.26%)	0.345 ± 0.069(81.58%)	0.175 ± 0.029(−7.9%)	0.26 ± 0.05(36.84%)
30	0.295 ± 0.0635(55.26%)	0.155 ± 0.016(−18.42%)	0.345 ± 0.071(81.58%)	0.185 ± 0.031(−2.63)	0.31 ± 0.074(63.16%)
40	0.365 ± 0.051(92.11%)	0.175 ± 0.017(−7.89%)	0.29 ± 0.086(52.63%)	0.22 ± 0.017(15.79%)	0.285 ± 0.045(50%)
50	0.35 ± 0.0585(84.21%)	0.165 ± (0.014(−13.16%)	0.245 ± 0.046(28.95%)	0.23 ± 0.024(21.05%)	0.31 ± 0.054(63.16%)

Results are expressed as means±SD (n = 3)
HFSD Hexane fraction of *Solanum dasyphyllum*, *DFSD* Dichloromethane fraction of *Solanum dasyphyllum*, *EAFSD* Ethyl Acetate fraction of *Solanum dasyphyllum*, *MFSD* Methanol fraction of *Solanum dasyphyllum*, *CFSD* Crude fraction of *Solanum dasyphyllum*
*percentage increase in activity as compared to toxicant

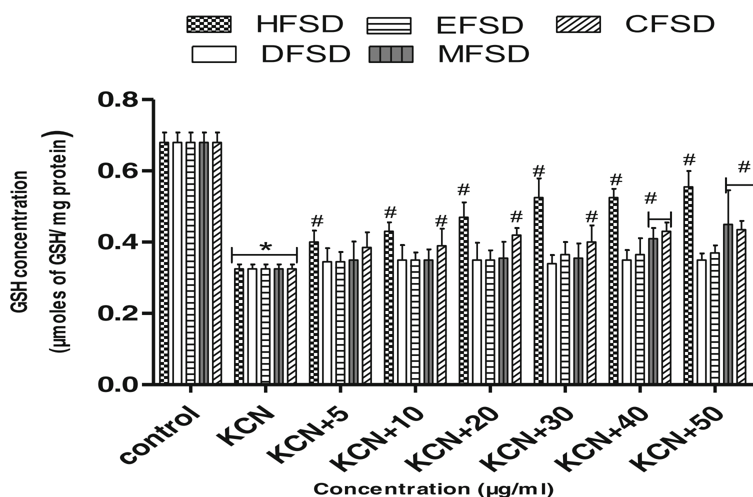

Fig. 1 Protective Effect of *Solanum dasyphyllum* on Glutathione in cerebrocortex of a rat challenged with potassium cyanide. Values are expresses as mean ± standard deviation. Values are expresses as mean ± standard deviation (*n* = 3). **P* < 0.05: Control vs KCN,#P < 0.05:KCN vs Treated. HFSD = Hexane fraction of *Solanum dasyphyllum*, DFSD = Dichloromethane fraction of *Solanum dasyphyllum*, EAFSD = Ethyl Acetate fraction of *Solanum dasyphyllum*, MFSD = Methanol fraction of *Solanum dasyphyllum*, SD = *Solanum dasyphyllum,* GSH = Glutathione

the fractions in slowing oxidative stress process and accumulation of their products in the brain, which may be one of the mechanisms by which the plants offer neuroprotection. One of the central activities of phytochemicals is in the mopping up of ROS, termination of chain reactions or donation of electrons to stop their reaction with macromolecules. There is no doubt that these antioxidant activities exemplified by various fractions of *S. dasyphyllum* play a significant role in the medicinal usage of the plant in the treatment of diseases. GSH is the major antioxidant molecule in the brain, which protects the brain against diverse pathogens [34], decreased GSH level is a distinguishing feature of KCN-exposed glial cells, and that deficiency of glutathione leads to pathological conditions in the brain

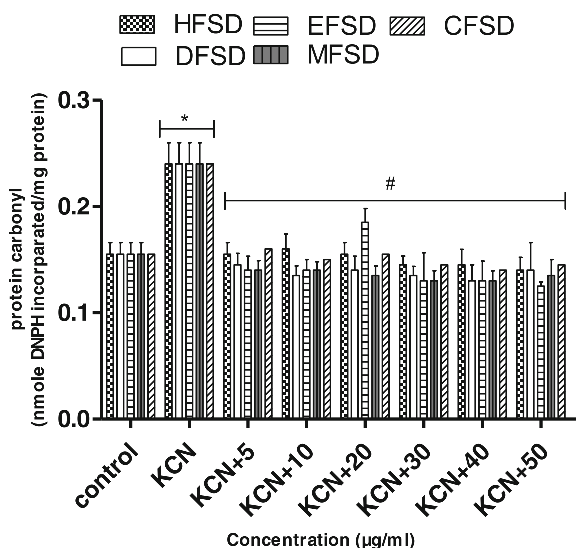

Fig. 2 Protective Effect of *Solanum dasyphyllum* on Protein carbonyl in cerebrocortex of a rat challenged with potassium cyanide. Values are expresses as mean ± standard deviation. Values are expresses as mean ± standard deviation (n = 3).*P < 0.05: Control vs KCN, #*P* < 0.05: KCN vs Treated. HFSD = hexane fraction of *Solanum dasyphyllum*, DFSD = Dichloromethane fraction of *Solanum dasyphyllum*, EAFSD = Ethyl Acetate fraction of *Solanum dasyphyllum*, MFSD = Methanol fraction of *Solanum dasyphyllum*, SD = *Solanum dasyphyllum,* PC=Protein carbonyl

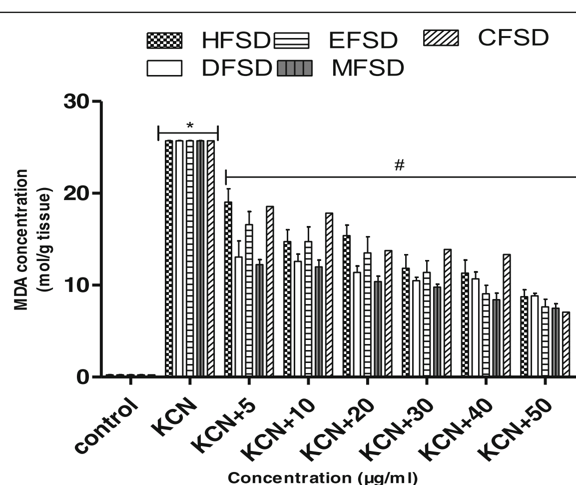

Fig. 3 Protective Effect of *Solanum dasyphyllum* on lipid peroxidation in cerebrocortex of a rat challenged with potassium cyanide. Values are expresses as mean ± standard deviation. Values are expresses as mean ± standard deviation (*n* = 3). *P < 0.05: Control vs KCN,#P < 0.05:KCN vs Treated. HFSD = hexane fraction of *Solanum dasyphyllum*, DFSD = Dichloromethane fraction of *Solanum dasyphyllum*, EAFSD = Ethyl Acetate fraction of *Solanum dasyphyllum*, MFSD = Methanol fraction of *Solanum dasyphyllum*. SD = *Solanum dasyphyllum.* MDA = Malonadialdehyde

[34]. In general, all the fractions were protective against KCN neurotoxicity, however, the extracts exhibited varying degrees of potency in some of the biochemical assays.

Conclusion

The outcome of this research suggested that all the solvent fractions of *S. dasyphylum* possess anticholinesterase activity, with mild antioxidant properties. Also observed is the preservation of mitochondria functions in the brain through maintenance of the activities of electron transport chain. These properties can be a reason for its usage as a panacea for snake poison and anticonvulsant. Further work can be done to identify and quantify the various bioactive compounds present in each solvent fraction and subject them to further neuroprotective studies.

Abbreviations

ATP: Adenosine Triphosphate; HFSD: Hexane Fraction *S. dasyphyllum.*; DFSDA: Dichloromethane Fraction *S. dasyphyllum*; EFSD: Ethylacetate Fraction *S. dasyphyllu.*; MFSD: Methanolic Fraction *S. dasyphyllum*; KCN: Potassium Cyanide; AChE: Acetylcholineterase; GSH: Reduced Glutathione; PC: Protein Carbonyl; LPO: Lipid Peroxidation; TBAR: TBA reactive substances; MDA: Malonedialdehyde; NCR: NADH-cytochrome c reductase; SCR: succinate cytochrome c reductase; NSD: NADH-succinate dehydrogenase; ROS: Reactive Oxygen Species; ETC: Electron Transport Chain

Acknowledgements

Not applicable.

Funding

Not applicable.

Authors' contributions

ACA, MTO and OBI designed and supervise the research, sample collection and experiment were conducted by OE, while the analysis of data and manuscript were done by OC and OBI. ACA and MTO were also involved in the interpretation of the result and editing of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors consent to the publication of the results.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 27 November 2017 Accepted: 14 February 2018
/ Published online: 09 April 2018

References

1. Abbott A. Solving the brain. Nature. 2013;499(18):272–4.
2. Stepien KM, Heaton R, Rankin S, Murphy A, Bentley J, Sexton D, Hargreaves IP. Evidence of oxidative stress and secondary mitochondrial dysfunction in metabolic and non-metabolic disorders. J. Clin Med. 2017;6(71):1–25.
3. Buckingham SD, Jones AK, Brown LA, Sattelle DB. Nicotinic Acetylcholine Receptor Signaling: Roles in Alzheimer's Disease and Amyloid, *Biotechnology. 2012:*327–35.
4. Venkatesan R, Ji E, Kim SY. Phytochemicals That Regulate Neurodegenerative Disease by Targeting Neurotrophins: A Comprehensive Review. BioMed Research International. 2015;814068:1–22.
5. Rani V, Deep G, Singh RK. Oxidative stress and metabolic disorders: pathogenesis and therapeutic strategies. Life Sci. 2016;148:183–93.
6. Carella F, Grassi MP, Savoiardo M, Contri P, Rapuzzi B, Mangoni A. Dystonic-parkinsonian syndrome after cyanide poisoning: clinical and MRI findings. Journal of Neurology Neurosurg Psychiatry. 1998;51:1345–8.
7. Aliyu BS. Common ethnomedicinal plants of the semi-arid regions of West Africa – their descriptions and phytochemicals. Triumph publishing company limited, Gidan Sa'adu Zungur, Kano, Nigeria. 2006;1:93–4.
8. Biller, J. Interface of neurology and internal medicine (illustrated Ed.). Lippincott Williams and Wilkins. 2007, p. 939.
9. Hariharakrishnan J, Satpute RM, Bhattacharya R. Cyanide-induced changes in the levels of neurotransmitters in discrete brain regions of rats and their response to oral treatment with a-ketoglutarate. Indian J Exp Biol. 2010;48:731–6.
10. Moneim AEA. Citrus Peel Extract Attenuates Acute Cyanide Poisoning-Induced Seizures. CNS Neurol Disord Drug Targets. 2014;13(4)
11. Shirwaikar⁎ A, Rajendran K, Bodla R, Dinesh Kumar C. Neutralization potential of *Viper russelli russelli* (Russell's viper) venom by ethanol leaf extract of *Acalypha indica*. J Ethnopharmacol. 2004;94:267–73.
12. Green CO, Wheatley AO, McGrowder DA, Dilworth LL, Asemota HN. Citrus peel polymethoxylated flavones extract modulates liver and heart function parameters in diet induced hypercholesterolemic rats. Food Chem Toxicol. 2013;51:306–9.
13. Leipnitz G, Amaral AU, Zanatta A, Seminotti B, Fernandes CG, Knebel LA, Vargas CR, Wajner M. Neurochemical evidence that phytanic acid induces oxidative damage and reduces the antioxidant defenses in cerebellum and cerebral cortex of rats. Life Science. 2010;87:275–80.
14. Ellman GL, Coutney KD, Andres V Jr, Feather-stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7:88–95.
15. Navarro A, Gomez C, Lopez-Cepero JM, Boveris A. Beneficial effects of moderate exercise on mice aging: survival, behavior, oxidative stress, and mitochondrial electron transfer. Am J Physiol Regul Integr Comp Physiol. 2004;286:505–11.
16. Ádám-Vizi V, Seregi A. Receptor independent stimulatory effect of noradrenaline on Na,K-ATPase in rat brain homogenate. Role of lipid peroxidation. Biochem Pharmacol. 1982;31:2231–6.
17. Rutter J. Succinate dehydrogenase –assembly, regulation and role in human disease. Mitochondrion. 2010;10:393–401.
18. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963;61:882–8.
19. Levine RL, Garland D, Oliver C, Amici A, Climent I, Lenz A. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol. 1990; 186:464–78.
20. Okhawa H. Assay for lipid peroxide in animal tissues by Thiobarbituric acid reaction. Annu Rev Biochem. 1979;95:351–2.
21. Adesina S.K. Constituents of Solanum Dasyphyllum fruit. J Nat Prod 1985; 48(1):147–147).
22. Joseph D, Muralidhara KM. Enhanced neuroprotective effectof fish oil in combination with quercetin against3-nitropropionic acid induced oxidative stress in rat brain. Prog Neuro-Psychopharmacol Biol Psychiatry. 2013;40:83–92.
23. Spinazzi M, Casarin A, Pertegato V, Salviati L, Angelini C. Assessment of mitochondrial respiratory chain enzymatic activities on tissues and cultured cells. Nat Protoc. 2012;7(6):1235–46.
24. Houghton P.J. and Howes J.R. the search for plants to manage neurodegenerative diseases. Ethnopharmacology (volume II). http://www.eolss.net/sample-chapters/c03/e6-79-17.pdf. Accessed 21 Nov 2017.
25. Hong HTK, Nose A, Agarie S. Oxidations of various substrate and effects of the inhibitors on purified mitochondria isolated from Kalanchoë pinnata. Biol Plant. 2005;49:201–8.
26. Rao SV, Muralidhara GM, Rajini PS. Neuromodulatory potential of aqueous extracts of cumin, cinnamon: evidence from rotenone model in drosophila: implications to Parkinson's disease. Int J Neurology Res. 2016;2(3–4):297–307.
27. Phom L, Achumi B, Alone DP, Muralidhara, Yenisetti SC. Curcumin's neuroprotective efficacy in drosophila model of idiopathic Parkinson's disease is phase specific: implication of its therapeutic effectiveness. Rejuvenation Res. 2014;17:481–9.

28. Venkatesan R, Ji E, Kim SY. Phytochemicals that regulate neurodegenerative disease by targeting Neurotrophins: a comprehensive review. Biomed Res Int. 2015:e814068.
29. Manjunath MJ, Muralidhara. Standardized extract of Withania Somnifera (Ashwagandha) markedly offsets rotenone-induced locomotor deficits, oxidative impairments and neurotoxicity in Drosophila Melanogaster. J Food Sci Technol. 2015;52:1971–81.
30. Li X-x, He G-r, Mu X, Xu B, Tian S, Yu X, Meng F-r, Xuan Z-h, Du G-h. Protective effects of baicalein against rotenone-induced neurotoxicity in PC12 cells and isolated rat brain mitochondria. Eur J Pharmacol. 2012;674:227–33.
31. Liu Z, Li T, Yang D, Smith WW. Curcumin protects against rotenone-induced neurotoxicity in cell and drosophila models of Parkinson's disease Advances in Parkinson's Disease. 2013;2:18–27.
32. Komolafe K, Akinmoladun AC, Komolafe TR, Olaleye MT, Akindahunsi AA, Joao BT. Rocha African locust bean (Parkia Biglobosa, Jacq Benth) leaf extract affects mitochondrial redox chemistry and inhibits angiotensin converting enzyme in vitro. Clinical Phytoscience. 2017;3(19):1–11.
33. Ilesanmi OB, Akinmoladun AC, Olayeriju OS, Saliu IO, Olaleye MT, Akindahunsi AA. Modulation of key biochemical markers relevant to stroke by antiaris africana leaf extract following cerebral ischemia/reperfusion injury. Afr J Tradit Complement Altern Med. 2017;14(4):253–64.
34. Allen J, Bradley RD. Effects of oral glutathione supplementation on systemic oxidative stress biomarkers in human volunteers. J Altern Complement Med. 2011;17(9):827–33.

Submit your manuscript to a SpringerOpen journal and benefit from:

- ► Convenient online submission
- ► Rigorous peer review
- ► Open access: articles freely available online
- ► High visibility within the field
- ► Retaining the copyright to your article

Submit your next manuscript at ► springeropen.com