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Hyperglycemia-induced oxidative brain injury: Therapeutic effects of *Cola nitida* infusion against redox imbalance, cerebellar neuronal insults, and upregulated Nrf2 expression in type 2 diabetic rats

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ABSTRACT

The therapeutic effect of the hot water infusion of *Cola nitida* against hyperglycemia-induced neurotoxicity, cerebellar neurodegeneration and elemental deregulations was investigated in fructose-streptozotocin induced rat model of type 2 diabetes (T2D). A diabetic group was administered drinking water, two other diabetic groups were treated with *C. nitida* at 150 and 300 mg/kg bodyweight respectively, while another group was administered metformin (200 mg/kg bodyweight). Two other groups consisting of normal rats, were administered drinking water and *C. nitida* (300 mg/kg bodyweight). After 6 weeks of treatment, their brains were collected. Treatment with *C. nitida* led to suppression of oxidative stress, significantly elevating reduced glutathione (GSH) levels, superoxide dismutase and catalase activities, concomitant with depletion of malondialdehyde (MDA) levels. Acetylcholinesterase and ATPase activities were significantly inhibited in *C. nitida*-treated diabetic rats. Histological and microscopic analysis also revealed a restorative effect of *C. nitida* on T2D-altered distribution of elements, neurons and axonal nodes. Treatment with *C. nitida* also led to significant inhibition of Nrf2 expression in the cerebellar cortex. These results suggest the therapeutic effects of *C. nitida* in maintenance of the neuronal integrity and antioxidant status of the brain in T2D. These neuroprotective activities can be attributed to the identified alkaloid, caffeine in the infusion.

1. Introduction

Neurodegeneration has been recognized amongst the many complications of type 2 diabetes (T2D), with the brain being the most affected organ (Toth, 2014). The susceptibility of the brain to T2D can attributed to its polyunsaturated fatty acids (PUFAs) constituents, which are target of peroxidative attack in oxidative stress. Oxidative stress arises in T2D owing to higher production of free radicals, outweighing the body's endogenous antioxidant system. This increase has been attributed to chronic hyperglycemia arising from persistent high blood glucose level due to insulin resistance and partial pancreatic β-cell dysfunction. Insulin resistance and pancreatic β-cell dysfunction are the main features of T2D and distinct it from type 1 diabetes (T1D), which occurs owing to the inability of the pancreatic β-cell to secrete insulin.

Inspite of high blood glucose level, glucose uptake in the brain has been reported to be altered in T2D owing to downregulation of glucose transporters at the blood-brain barrier (BBB) (Gejl et al., 2017; Hwang et al., 2017; Pardridge et al., 1990). This alteration can also be a major contributor to brain damage in T2D, as the brain is glucose-dependent for its functions.

The costs of treating diabetes mellitus (DM) and its complications are of tremendous concern to health practitioners, particularly in developing countries with poor health infrastructure and very low gross domestic products (GDP) (Erukainure et al., 2017a, 2018). Hence, the search for cheaper alternatives with little or no side effects and high efficacy have led a paradigm shift to natural medicines, with medicinal plants playing an influential role. Medicinal plants have been reported for their roles in the folkloric treatment of several ailments such as DM, malaria, tuberculosis and neurodegeneration.

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Cola nitida is amongst the common medicinal plants employed in the folkloric treatment of several ailments, such as depression, parasitic infections, asthma, rheumatism, whooping coughs, and low libido (Asogwa et al., 2011; Dorathy et al., 2014; Ezuruike and Prieto, 2014; Ndagi et al., 2012). It is an evergreen tree belonging to the genus, *Cola* and commonly called kola nut. It is commonly chewed as stimulant. Its utilization in religious, social, and ceremonial functions have been reported (Asogwa et al., 2011; Ndagi et al., 2012). Its antidiabetic and antioxidant properties have been demonstrated *in vitro* and *in vivo* (Ayebe et al., 2012; Erukainure et al., 2017b; Oboh et al., 2014). Its consumption with alcohol has been reported to modify neuronal processes (Obochi et al., 2009). Its influence on the etiology of malaria-morbidity have been reported (Alaribe et al., 2003). Its chronic consumption has been shown to have little or no significant effect on locomotor activities (Umoren et al., 2009). These activities can be attributed to the reported phytochemical constituents of *C. nitida*, which includes caffeine, kolanin, L-epicatechin, and d-catechin, theophylline, quinine, and theobromine (Erukainure et al., 2017b; Lowor et al., 2010; Obochi et al., 2009).

This study aimed at investigating the therapeutic effect of *C. nitida* hot infusion on oxidative imbalance, ATPase and cholinergic enzyme activities in whole brain of type 2 diabetic rats. The cerebellar cortices were also investigated for morphological changes, neuronal and elemental distribution, as well as Nrf2 expression, the master regulator of antioxidant activity.

2. Materials and methods

2.1. Plant material

C. nitida were purchased at Ifon, Nigeria. They were identified and authenticated at the Department of Botany, University of Benin, Benin City, Nigeria. They were assigned the voucher specimen number, UBH_C 323 and deposited at the herbarium.

The seeds were air dried before blending and storage in zip-lock bags for subsequent analysis.

2.2. Extraction

About 200 g of the blended sample was infused in boiling distilled water and allowed to stand overnight. The extract was decanted and concentrated at 50 °C in a water bath. The resulting concentrate was stored in glass vials for subsequent analysis.

2.3. Experimental animals

Thirty-eight male albino rats (Sprague-Dawley) weighing 180–200 g were obtained from the Biomedical Research Unit (BRU), University of KwaZulu-Natal, Durban, South Africa. The rats were adapted for 7 days and maintained under optimum temperature and humidity with natural photo period of 12-h light-dark cycle. Food and water were provided *ad libitum*.

The study was carried out in accordance with the approved guidelines of the Animal Ethics Committee, University of KwaZulu-Natal, Durban, South Africa (protocol approval number: AREC/020/017D).

2.4. Groupings

The study consisted of treatment groups: **Normal Control (NC)**: Normal rats (non-diabetic and not treated); **Diabetic Control (DC)**: Diabetic and untreated control; **Diabetic + low dose (DLDC)**: Diabetic rats administered low dose (150 mg/kg bodyweight [bw]) of *C. nitida* infusion; **Diabetic + high dose (DHDC)**: Diabetic rats administered high dose (300 mg/kg bw) of *C. nitida* infusion; **Diabetic + standard drug metformin (DSM)**: Diabetic rats administered 200 mg/kg bw metformin; and **Normal Toxicological Group (NTXC)**: Non-diabetic rats administered high dose of *C. nitida* infusion.

NC and NTXC constituted the normal groups with 5 rats each. While DC, DLDC, DHDC, and DSM constituted the diabetic groups with 7 rats each.

2.5. Induction of type 2 diabetes

For 2 weeks, 10% fructose solution was supplied *ad libitum* to the diabetic groups (DC, DLDC, DHDC, and DSM) instead of drinking water. Then they were fasted overnight and injected intraperitoneally (i.p) with STZ (40 mg/kg BW) dissolved in citrate buffer (pH 4.5). The normal groups were supplied with drinking water *ad libitum* and injected (i.p) with citrate buffer only.

The non-fasting blood glucose (NFBG) levels of all rats were checked with a glucometer (Glucoplus, Quebec, Canada) seven days later. Rats with blood glucose level > 200 mg/dL were considered as diabetic.

2.6. Intervention trial

The animals in DLDC and DHDC groups were orally administered with *C. nitida* infusion at low and high doses respectively with the aid of a gastric gavage needle. The animals in NTXC group were also orally administered with high dose of the infusion. Distilled water was administered to the animals in NC and DC groups. These treatments were carried out once a day for five days a week, while the other two days were used as a period for the recovery from stress associated with gastric gavage. The intervention period lasted for 6 weeks.

2.7. Sacrifice and collection of brains

At the end of the intervention period, the rats were humanely sacrificed by euthanizing with halothane anesthesia. The brains were collected and washed in 0.9% NaCl and weighed. Parts of the cerebellum were excised and fixed in 10% neutral buffered formalin for histology and immunohistochemistry. For electron microscopy, about 2 mm of the cerebellum was excised and fixed in 2.5% glutaraldehyde. The remaining parts of the brain were homogenized in 50 mM sodium phosphate buffer (with 10% triton X-100, pH 7.5), and centrifuged at 15,000 rpm at 4 °C for 10 min. The supernatants were collected and stored at −20 °C for subsequent studies.

2.8. Determination of oxidative stress and proinflammatory biomarkers

The supernatants were analyzed for oxidative stress biomarkers and antioxidant activities by determining the reduced glutathione (GSH) level (Ellman, 1959), catalase (Chance and Maehly, 1955) and superoxide dismutase (SOD) (Kakkar et al., 1984) activities, and malondialdehyde (MDA) level (Chowdhury and Soulsby, 2002). In order to determine the level of proinflammation, the supernatants were analyzed for myeloperoxidase activity (Granell et al., 2003).

2.9. Determination of ATPase activity

The supernatant were analyzed for ATPase activities using previously described protocol (Adewoye et al., 2000; Erukainure et al., 2017a).

2.10. Determination of acetylcholinesterase activity

The acetylcholinesterase activities of the brain tissues were determined by the Ellman's protocol (Ellman et al., 1961).

2.11. Histology

Following embedding and sectioning to 4 μm in slides, the fixed cerebellar tissues were subjected to hematoxylin and Nissl staining for

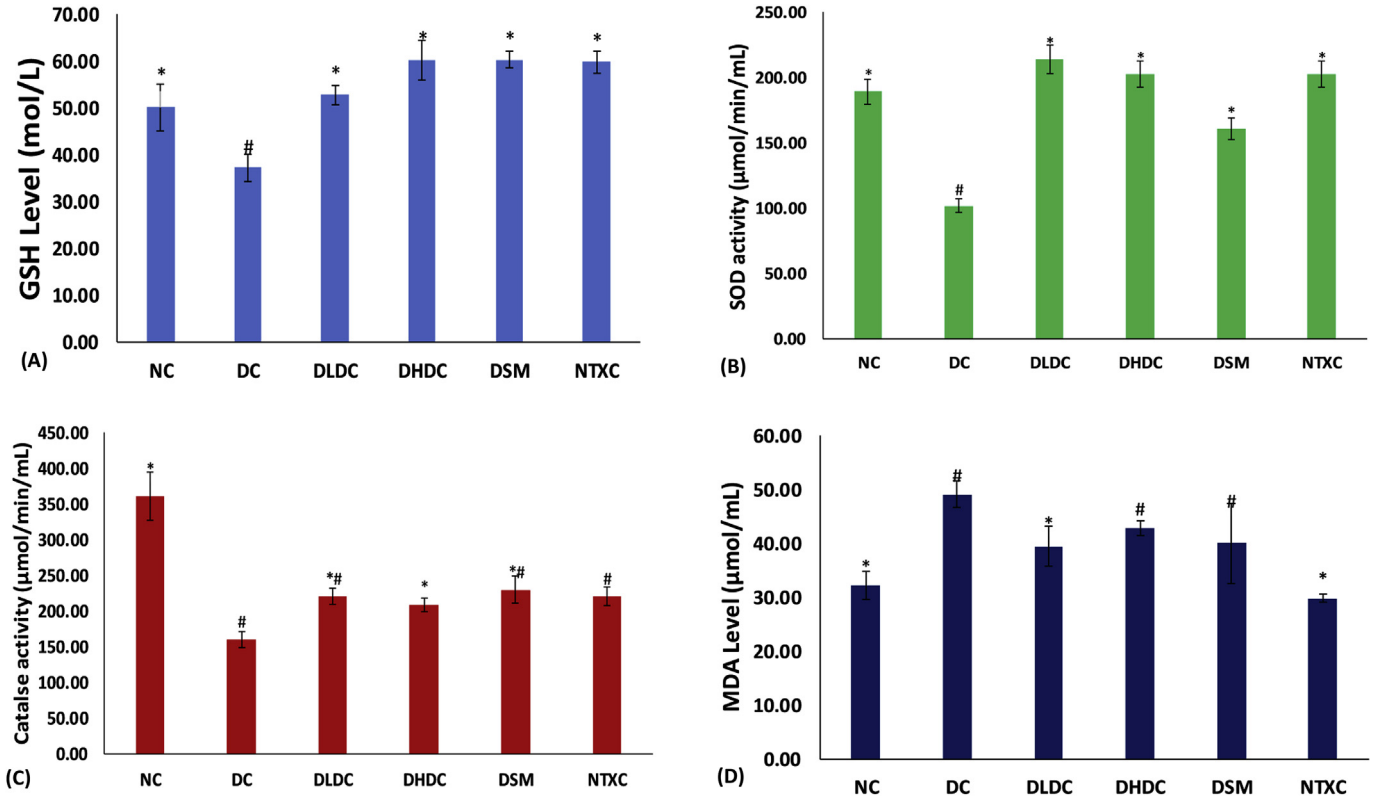

Fig. 1. Antioxidant status of experimental groups. Values = mean ± SD; n = 5 (NC and NTXC) and 7 (DC, DLDC, DHDC, and DSM). *Statistically ($p < 0.05$) significant to DC, #statistically significant ($p < 0.05$) to NC.

5 min and rinsed with tap water. The slides were thereafter stained with eosin. The slides were cover-slipped after mounting in DPX. Images were viewed with a Leica slide scanner (SCN 4000, Leica Biosystems, Germany).

2.12. Scanning electron microscopy

A scanning electron microscope (SEM; Zeiss Ultra Plus) was utilized for microscopic analysis of the cerebellar cortex. After buffer-washing thrice at 5 min intervals, 0.5% osmium tetroxide was used in post-fixing the tissues for 2 h. The tissues were subjected to dehydration with ethanol of increasing concentrations: 25% (twice at 5 min interval), 50% (twice at 5 min interval), 75% (twice at 5 min interval) and 100% (twice at 10 min interval) after washing with distilled water thrice at 5 min interval. The dehydrated samples were dried in a critical-point-dryer apparatus and then gold coated. Images were taken at an accelerating voltage of 20–25 k.

2.13. Energy dispersive X-ray (EDX) microanalysis

Following SEM analysis, the gold-coated samples were subjected to energy dispersive X-ray (EDX) microanalysis using a SEM (Zeiss Ultra Plus) equipped with an Oxford Instruments X-Max 80 mm^2 Solid State EDX detector in order to determine the elemental levels.

2.14. Immunohistochemistry

Thin sections of 5 µm thickness were obtained from routine paraffin embedded tissues prepared during histology. Following deparaffinization, sections were subjected to antigen retrieval by heating in a citrate-based antigen unmasking solution, pH 6.0 (Vector®, Burlingame, CA, USA; #H3300) for 30 min in a steamer and cooled on the bench at room temperature for 30 min. Endogenous peroxidase blocking was performed in 0.3% hydrogen peroxide in Tris Buffered Saline (TBS, pH 7.4) for 10 min. Sections were blocked in 2.5% normal horse serum (Vector® #MP-7401) for 20 min. Sections were then incubated overnight at 4 °C in primary rabbit antibodies: Nrf2 (Abcam, MA, USA; #ab31163) at 1:100 dilution. Sections were washed in TBS and incubated in ImmPRESS™ HRP Anti-Rabbit IgG (Peroxidase) Polymer Reagent, made in horse (Vector® #MP-7401). Colour was developed with DAB Peroxidase (HRP) Substrate Kit (Vector® #SK-4100), and sections were counter-stained in Harris hematoxylin. Sections without primary antibodies were similarly processed to control for binding of the secondary antibody. No specific immunoreactivity was detected on these control sections. Immunostained sections were digitized with a Pannoramic 250 Flash II slide scanner (3D Histech, Budapest, Hungary) and representative photomicrographs were obtained at 200× magnification using 3DHistech CaseViewer Software. Image analysis was performed using ImmunoRatio plugin on Image J software (NIH, USA), which separates and quantifies the percentage of DAB (positive immunoreactivity) by digital colour deconvolution (Tuominen et al., 2010).

2.15. Gas chromatography-mass spectrometric (GC-MS) analysis

To identify the bioactive compound/s that may be responsible for the neuroprotective activities of *C. nitida* hot infusion, the infusion was subjected to GC-MS analysis. This was carried out via an Agilent technologies 6890 Series GC coupled with (an Agilent) 5973 Mass Selective detector, driven by Agilent Chemstation software. A HP-5MS capillary column d (30 m × 0.25 mm ID, 0.25 µm film thickness, 5% phenylmethylsiloxane) was utilized. The carrier gas was an ultra-pure helium and at a flow rate of 1.0 mL min^{-1} and a linear velocity of 37 cm s^{-1}. Other working conditions were: Programmed oven temperature: 280 °C (at the rate of 10 °C min^{-1} with a hold time of 3 min); Injector temperature: 250 °C; Initial oven temperature: 60 °C; Electron

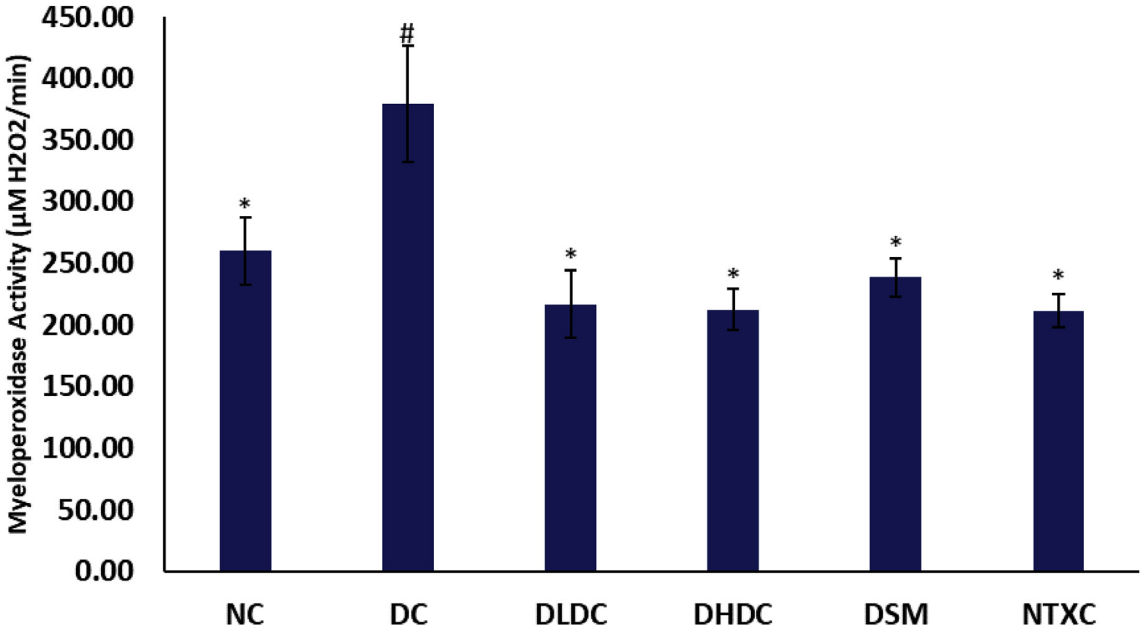

Fig. 2. Myeloperoxidase activities of experimental groups. Values = mean ± SD; n = 5 (NC and NTXC) and 7 (DC, DLDC, DHDC, and DSM). *Statistically ($p < 0.05$) significant to DC, #statistically significant ($p < 0.05$) to NC.

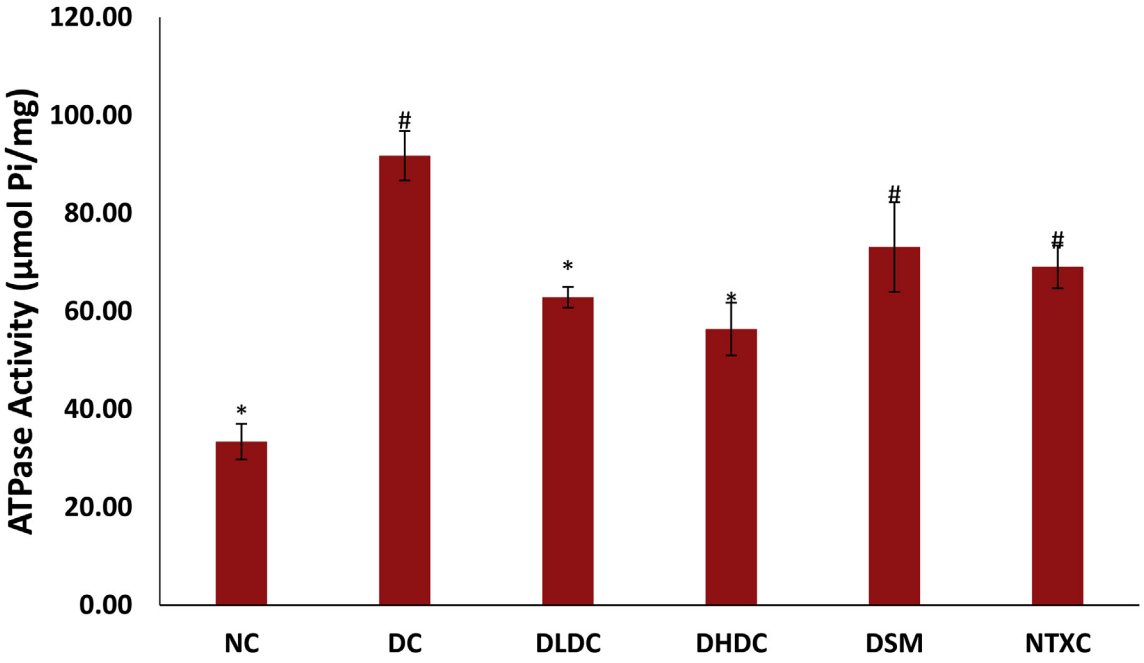

Fig. 3. ATPase activities of experimental groups. Values = mean ± SD; n = 5 (NC and NTXC) and 7 (DC, DLDC, DHDC, and DSM). *Statistically ($p < 0.05$) significant to DC, #statistically significant ($p < 0.05$) to NC.

ionization mode: 70 eV; electron multiplier voltage: 1859 V; Ion source temperature: 230 °C; Quadrupole temperature: 150 °C; Solvent delay: 4 min; Scan range: 50–70 amu. An inbuilt NIST mass spectral library was used in identifying the compounds.

3. Statistical analysis

Data obtained were subjected to one-way analysis of variance (ANOVA) and significant differences were established at $p < 0.05$, with IBM SPSS for Windows, version 23.0 (SPSS Inc., Chicago, IL) and Microsoft (2016) Excel Spreadsheet. Data from immunoreactivity quantification were analyzed with GraphPad Prism 5 (GraphPad Inc. San Diego, US). Results were reported as mean ± SD.

4. Results

As depicted in Fig. 1, there was a significant ($p < 0.05$) decrease in GSH level (Fig. 1A), SOD and catalase activities (Fig. 1B and 1C), with concomitant elevation in MDA level (Fig. 1D) upon induction of T2D. These were significantly ($p < 0.05$) reversed in the treatment groups, with *C. nitida* displaying a favourable antioxidant activity when compared to metformin.

Induction of T2D led to an increased myeloperoxidase activity as shown in Fig. 2, consistent with a proinflammatory effect. Treatment with both doses of *C. nitida* led to significant ($p < 0.05$) reduction reaching levels indistinguishable from the normal controls (NC).

The ATPase activity was significantly ($p < 0.05$) elevated on

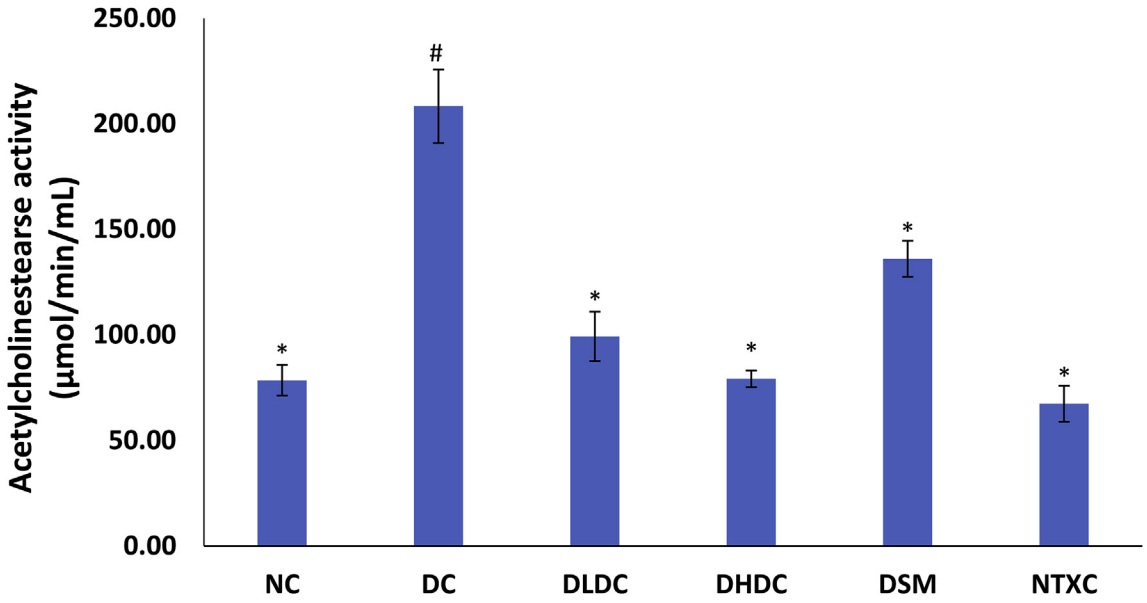

Fig. 4. Acetylcholinesterase activities of experimental groups. Values = mean ± SD; n = 5 (NC and NTXC) and 7 (DC, DLDC, DHDC, and DSM). *Statistically ($p < 0.05$) significant to DC, #statistically significant ($p < 0.05$) to NC.

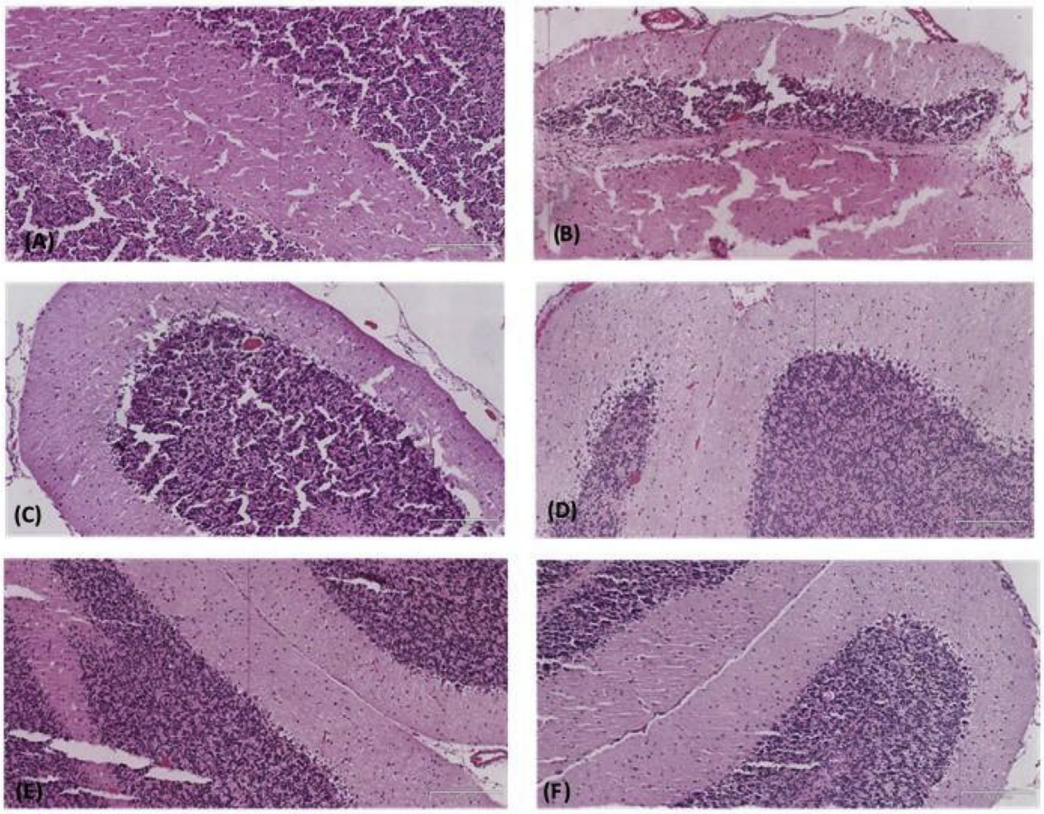

Fig. 5. Morphological changes in cerebellar tissues of experimental groups. Magnification: 10×. (A) = NC, (B) = DC, (C) = DSG-LD, (D) = DSG, (E) = DSM, and (F) = PHD.

induction of T2D as shown in Fig. 3. This was significantly ($p < 0.05$) reduced in rats treated with *C. nitida* to levels insignificant from the normal control.

There was a significant ($p < 0.05$) elevation of acetylcholinesterase activity on induction of T2D as depicted in Fig. 4. This was significantly ($p < 0.05$) reduced in all treatment groups to levels indistinguishable from the normal controls, with *C. nitida*-treated rats showing more inhibitory effect compared to metformin.

As shown in Fig. 5, induction of T2D led to altered morphology of the cerebellar cortex as portrayed by inflamed and distorted Purkinje and granular cell layers (Fig. 5B), indicating consistent with neuronal insult and compromised integrity as compared to that of the normal control (Fig. 5A). Intact morphology indistinguishable from the normal control (NC) was observed for all treatment groups (Fig. 5C–E), with

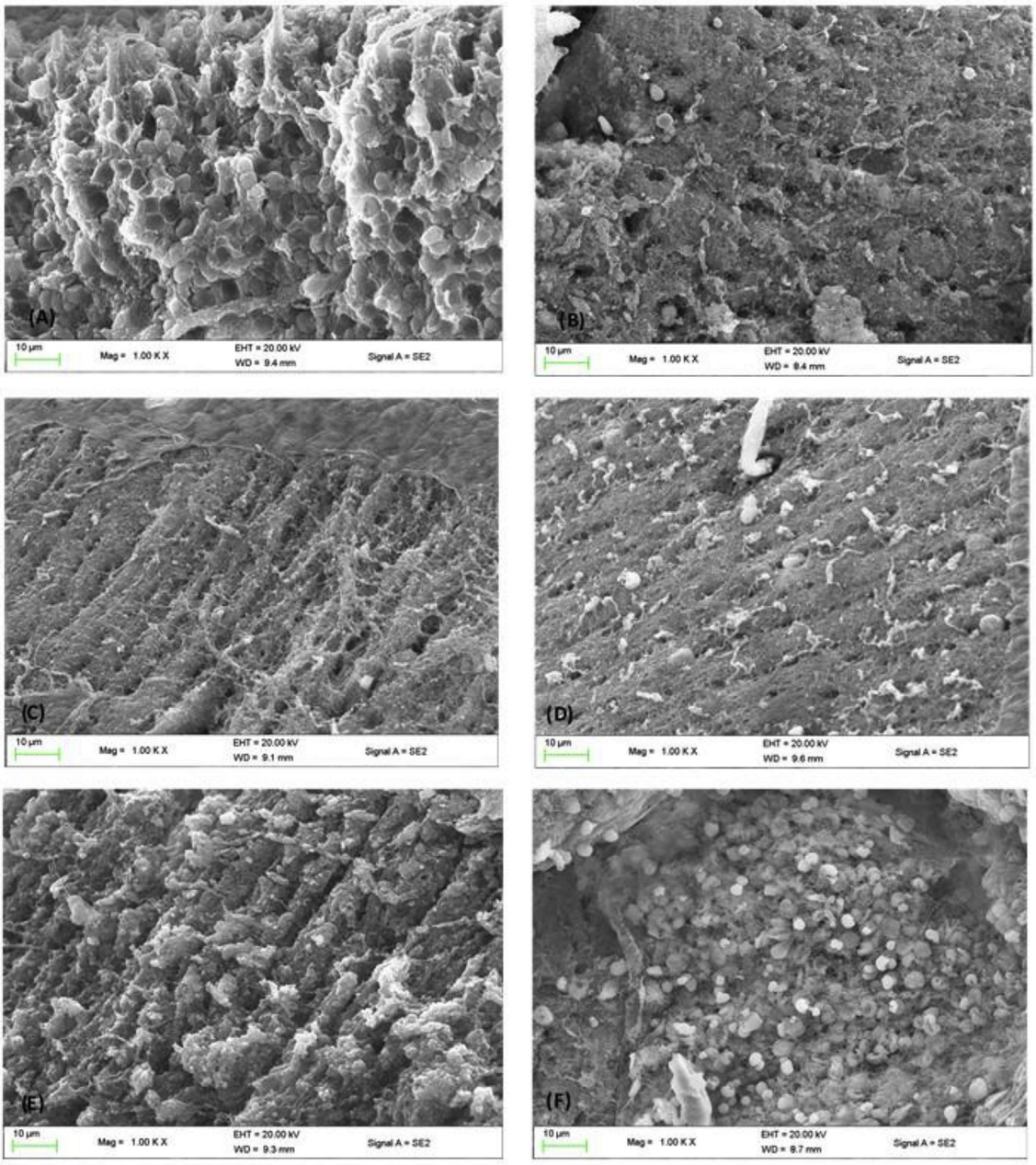

Fig. 6. SEM analysis of cerebellar tissues of experimental groups. Magnification = 1000×. (A) = NC, (B) = DC, (C) = DSG-LD, (D) = DSG, (E) = DSM, and (F) = PHD.

rats treated with high dose of *C. nitida* showing a more intact morphology (Fig. 5F).

As shown in Fig. 6, the cerebellar cortex of the normal rats consists of intact Purkinje and glial cells, with vast networks of dendrites and axons (Fig. 6A) as revealed by SEM analysis. This intactness was however distorted on induction of T2D as depicted by the depleted number of Purkinje and glial cells, with concomitant alterations in the dendrites and axons network (Fig. 6B). Treatment with low dose of *C. nitida* led to improve network of dendrites and axons, but showed little or no improvement on the numbers of Purkinje and glial cells (Fig. 6C). These were more improved in rats treated with high dose of *C. nitida* (Fig. 6D). Administration of *C. nitida* to normal rats increased the population of Purkinje and glial cells in the cerebellar cortex, with discernible effect on the dendrites and axons networks (Fig. 6F).

Quantitative analysis of the of EDX mapping of the cerebellar cortex revealed depleted levels of carbon, nitrogen, iron, and potassium with concomitantly elevated levels of oxygen, sodium, aluminum and calcium as depicted in Fig. 7A and 7B. Treatment with *C. nitida* had no discernible effects on the carbon and oxygen levels, but led to the reversal of the other elements to levels indistinguishable from the normal controls. Treatment with metformin led to the presence of cobalt in cerebellar cortex of rats with T2D (Fig. 7A and 7B).

Nrf2 expression is observed in cells nuclei and cytoplasm; particularly increased nuclear expression is observed in Purkinje neurons following diabetic induction (Fig. 8A). Quantification of immunoreactivity showed a significant ($p < 0.05$) exacerbated expression of Nrf2 in the cerebellar cortex on induction of T2D as depicted in Fig. 8B. The expressions were significantly ($p < 0.05$) attenuated in the cerebellar cortexes of the treatment groups to levels indistinguishable from the normal control.

GC-MS analysis of *C. nitida* hot infusion revealed the presence of caffeine only as shown in Fig. 9, indicating the near purity of the infusion.

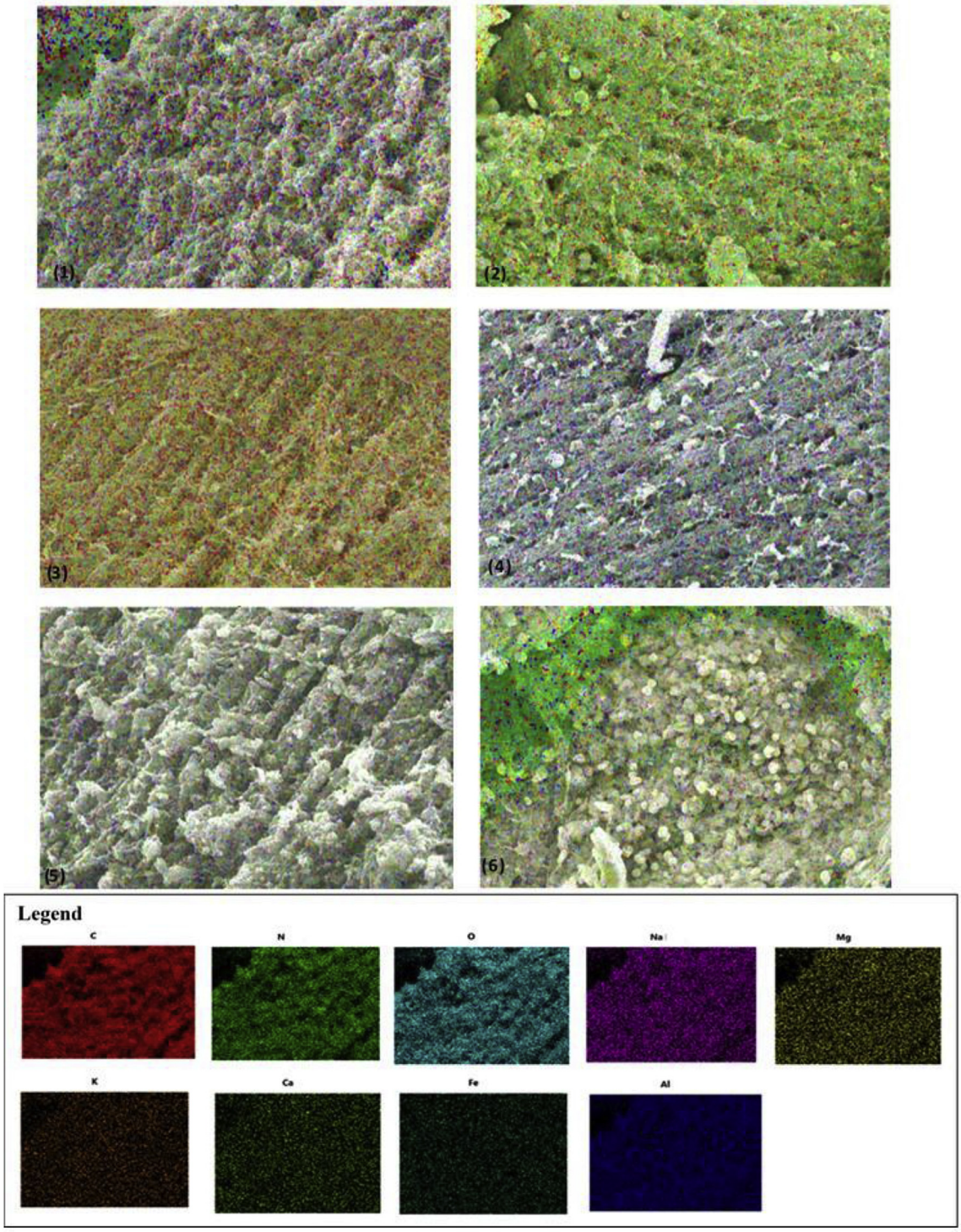

Fig. 7A. EDX mapping of cerebellar tissues of experimental groups. Magnification = 1000 ×. (1) = NC, (2) = DC, (3) = DLDC, (4) = DHDC, (5) = DSM, and (6) = NTXC. Legends: C = carbon, N = nitrogen, O = oxygen, Na = sodium, Mg = magnesium, K = potassium, Ca = calcium, Fe = iron, and Al = aluminum.

5. Discussion

Alterations in glucose homeostasis owing to suppressed glucose transportation across the BBB as well as the susceptibility of the brain's PUFAs to oxidative damage have been recognized as critical players in the pathogenesis and progression of neurodegeneration in T2D (Gejl et al., 2017; Hwang et al., 2017; Kim et al., 2015; Uttara et al., 2009). These alterations and oxidative damage are often characterized by morphological changes, neuronal loss and metal accumulation (Folarin et al., 2017; Kim et al., 2015; Wrighten et al., 2009). The neuroprotective effect of several medicinal plants and their constituents has been reported, with antioxidative stress serving as a major neuroprotective mechanism (Bhattacharya et al., 2000; Kumar, 2006; Uddin et al., 2013). Although there are conflicting reports on the clinical efficacies of antioxidants in combating oxidative stress (Zhou et al., 2018), studies have however shown that most antioxidants may exhibit their therapeutic effect by blocking the generation of free radicals rather than scavenging (Johansen et al., 2005). In the present study, the

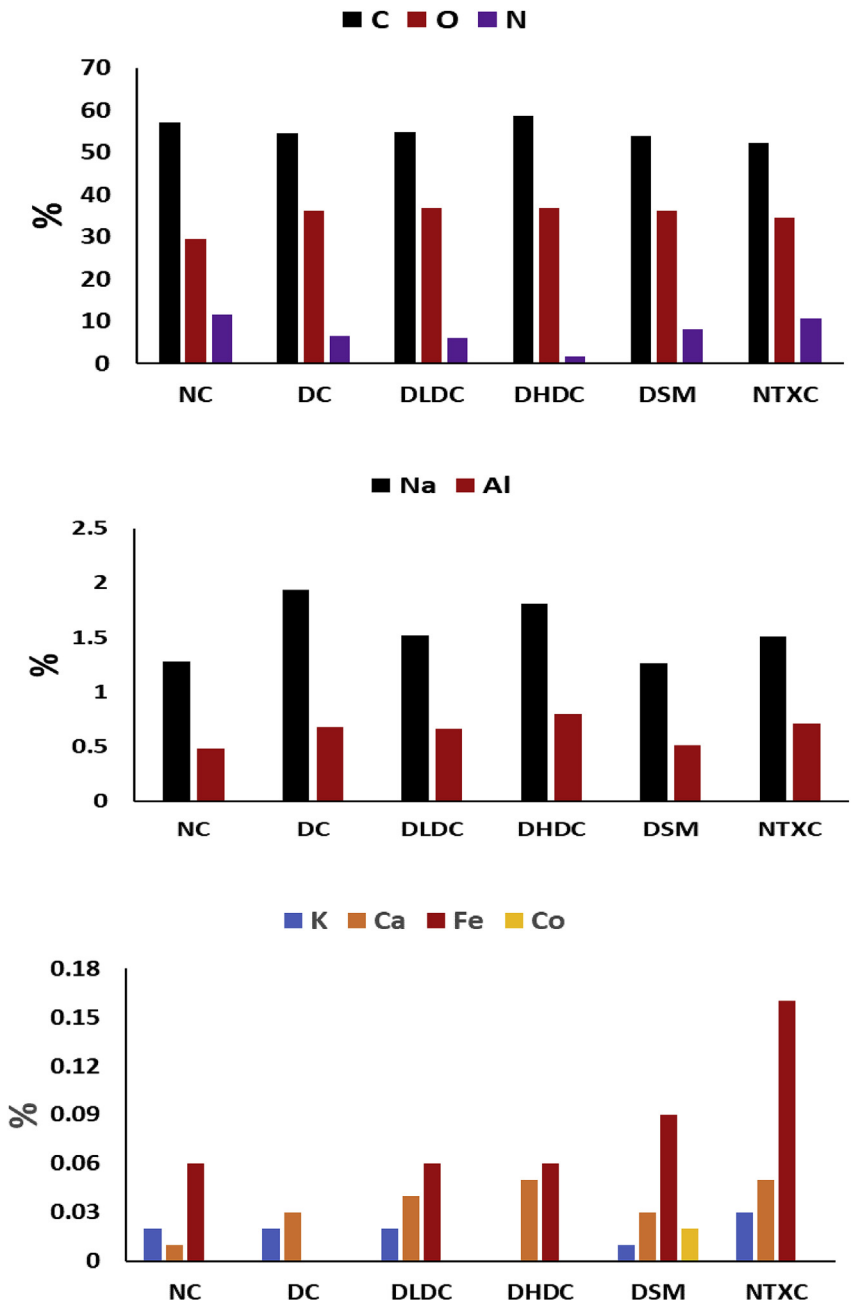

Fig. 7B. Quantitative analysis of EDX mapping of cerebellar tissues of experimental groups. Legends: C = carbon, N = nitrogen, O = oxygen, Na = sodium, Co = cobalt, K = potassium, Ca = calcium, Fe = iron, and Al = aluminum.

neuroprotective effect of caffeine-rich infusion from *C. nitida* on neuropathology of the brain in T2D was investigated.

The brain has a relatively low endogenous antioxidant system, which when coupled with its high PUFAs content, the excitotoxic and auto-oxidizable activities of neurotransmitters increases its susceptibility to oxidative stress (Patel, 2016). In the present study, the depleted GSH level, SOD and catalase activities in the untreated diabetic rats ($p < 0.05$) corroborates underlying oxidative stress status, which can be attributed to hyperglycemia-induced generation of ROS. This is in agreement with previous studies on the depleted level and activities of these antioxidants in brains of diabetic rats (Mastrocola et al., 2005; Moreira et al., 2003). The generation of ROS, such as $O_2\cdot^-$ and ·OH are toxic to neuronal cells and their production from persistent glucose oxidation have been reported (Maritim et al., 2003). Glucose in its enediol form, undergoes oxidation in a transition-metal-dependent reaction to an unstable enediol radical anion. Owing to its instability, the anion is converted to reactive ketoaldehydes and $O_2\cdot^-$ (Maritim et al., 2003). SOD dismutates the generated $O_2\cdot^-$ to H_2O_2, which if not acted on by catalase will be further broken down to give ·OH. Both H_2O_2 and ·OH can switch on the lipid peroxidation process, with PUFAs being the most susceptible owing to their double bonds. An initiation of lipid peroxidation due to hyperglycemia is portrayed in the present study by the elevated MDA level in the untreated diabetic rats (Fig. 1D). H_2O_2 is also involved in the proinflammatory cascade as it can be converted to hypochlorite (HOCl) in the presences of myeloperoxidase and hydrochloric acid (HCl) (Patel, 2016). Thus, the reversed levels of GSH and MDA, as well as SOD, catalase and myeloperoxidase activities in rats treated with *C. nitida* (Figs. 1–2) may depict a reduction in the generation of free radicals, which portrays an antioxidative and anti-proinflammatory protective effect of the infusion

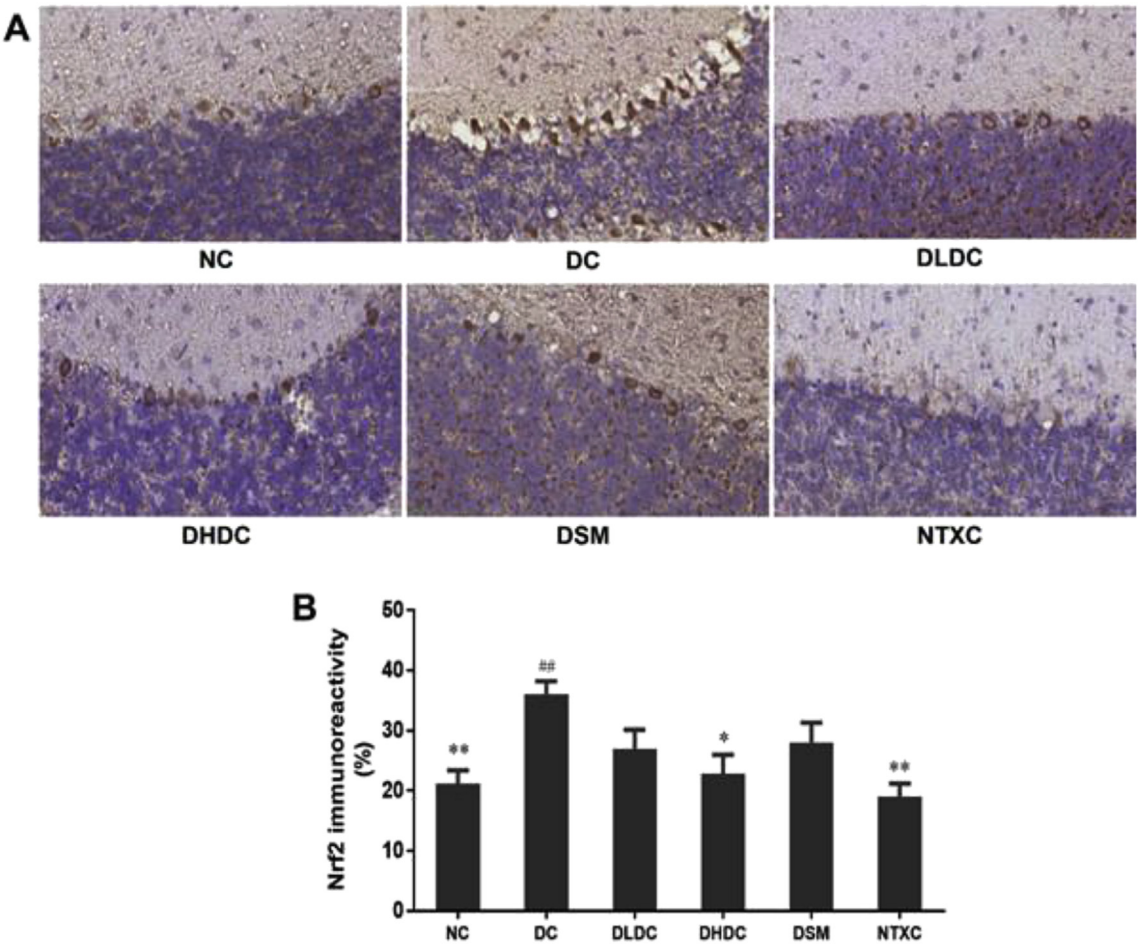

Fig. 8. (A): Nrf2 expression in the cerebellum of experimental rats evaluated by immunohistochemistry; Mag = 400 ×. (B): Image J analysis of nrf2 immunoreactivity. *P < 0.05, **P < 0.01 compared to DC; ##P < 0.01 compared to control.

against hyperglycemia-induced oxidative brain.

Increased ATPase activity leading to impaired facilitative transportation of glucose across the BBB and altered levels of ATP have been reported in neurodegenerative diseases (Falkowska et al., 2015; Magistretti and Allaman, 2015; Mochel et al., 2012; Zhang et al., 2015). The increased ATPase activity in brains of untreated diabetic rats (Fig. 3) may thus indicate an impaired glucose transportation on induction of T2D and depleted ATP level. The reduced ATPase activity in brains of rats treated with *C. nitida* portrays the ability of the infusion to inhibit hyperglycemia-induced ATPase alteration, which may also reflect an increased facilitative glucose uptake in the brain. This corroborates previous reports on the suppressive effects of *C. nitida* on ATPase activities in brain tissues of STZ-induced diabetic rats (Imam-Fulani et al., 2018).

The hydrolyzing effect of acetylcholinesterase on the neurotransmitter, acetylcholine has been linked with the pathogenesis and progression of neurodegenerative diseases (Kuhl et al., 1999; Mushtaq et al., 2014; Pavlov et al., 2009). Impaired cognition, and motor neuron dysfunction are amongst the neurodegenerative diseases linked to increased acetylcholinesterase activities (Hwang et al., 1999; Kuhad et al., 2008; Tabet, 2006). The increased activity on induction of T2D (Fig. 4) is in consent with reports on increased acetylcholinesterase activities in diabetic rat brains (Ghareeb and Hussen, 2008; Kuhad et al., 2008), further indicating a hyperglycemia-induced brain injury. The reduced activities in the brains of rats treated with *C. nitida* portrays a neuroprotective potential of the infusion, which can be attributed to the caffeine constituent (Fig. 9). This corroborates previous reports on the inhibitory effect of *C. nitida* and caffeine on acetylcholinesterase activities (Oboh et al., 2018; Salahdeen et al., 2014).

The role of cerebellum in the facilitation of movement, motor and muscular activities is well documented (Imosemi, 2013; Popescu et al., 2009). Purkinje cells have been recognized as the major neurons present in the cerebellum (Fahrion et al., 2013; Lopez et al., 2009). Morphological changes in the brain have been implicated in most neurodegenerative diseases, and has been linked to oxidative stress (Ojo et al., 2014; Sidhu and Nehru, 2004). The distorted layers of Purkinje and granular cells in cerebellar cortexes of the untreated diabetic rats (Fig. 5B) depict morphological alterations. This is further obvious in the depleted Purkinje and glial cells as well as distorted networks of dendrites and axons (Fig. 6B). These morphological changes insinuate a compromised neuronal integrity and can be attributed to hyperglycemia-induced oxidative stress (Fig. 1A) as Purkinje cells have been reported for their vulnerability to oxidative stress (Chen et al., 2003; Kern and Jones, 2006; Lopez et al., 2009). These alterations correlate with previous reports on structural changes in the cerebellar cortexes of diabetic rats (Hernández-Fonseca et al., 2009; Ozdemir et al., 2016). Restoration of the layers and improved networks of the dendrites and axon in cerebellar cortexes of *C. nitida*-treated diabetic rats (Fig. 5(C and D) and 6 (C and D)) imparts further evidence for the neuroprotective effect of the infusion. This activity can be attributed to its caffeine constituent as the alleviative effect of caffeine on the loss of Purkinje neurons have been reported (Gonçalves et al., 2017).

The roles of elements, particularly metals in the normal functions of the brain has been reported. They often act as co-factors for enzymes and are involved in multiple redox reaction (Uttara et al., 2009).

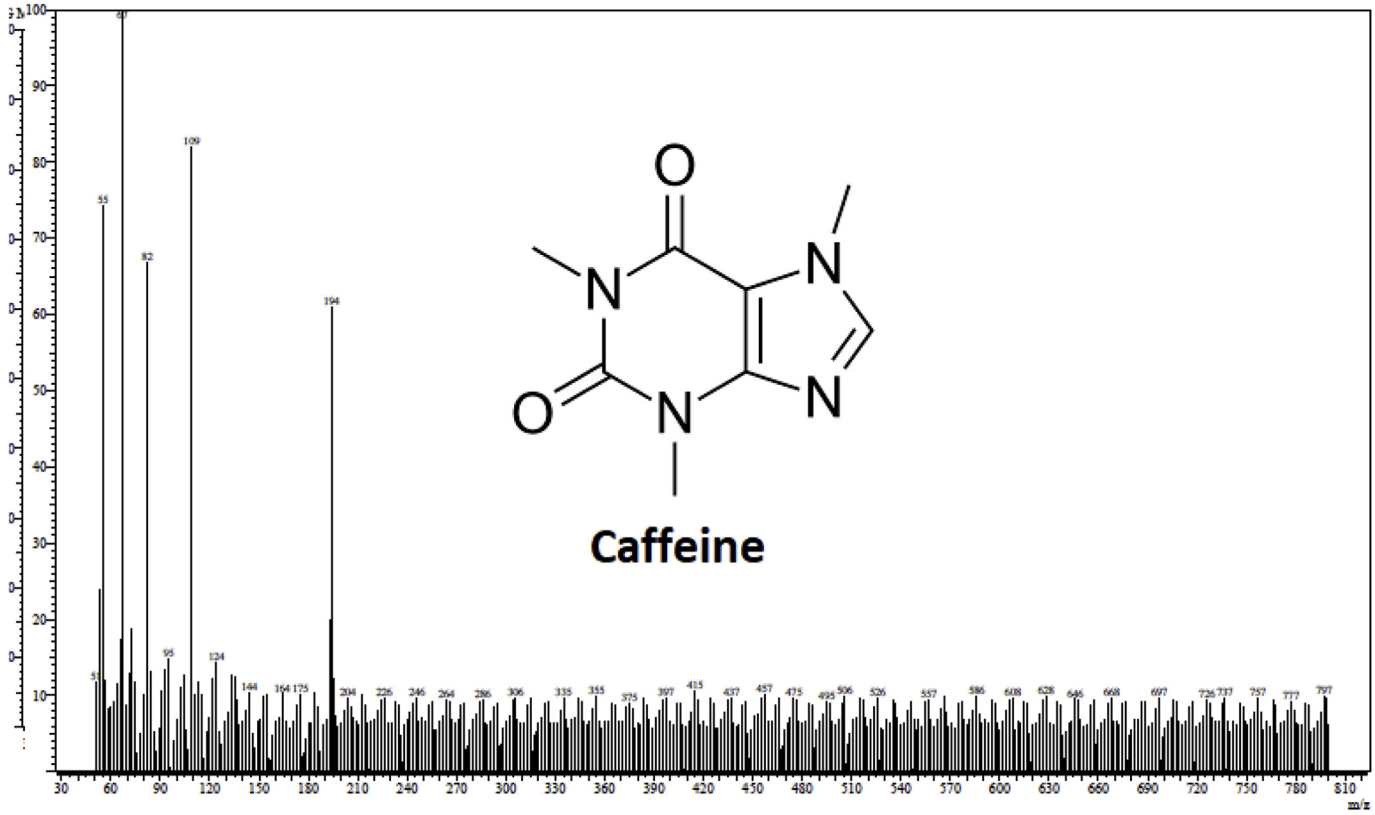

Fig. 9. MS spectra of GC-MS identified compound in *C. nitida* hot infusion.

Alterations in their distribution have been linked with neurodegeneration, particularly cerebellar toxicity (Folarin et al., 2017; Kamal and Kamal, 2013). The altered elemental levels (Fig. 7A and 7B), particularly elevated levels of oxygen, aluminum and sodium in the cerebellar cortexes of the untreated diabetic rats may indicate toxicity as their accumulations have been reported in neurodegeneration (Kamal and Kamal, 2013; Wojda et al., 2008). The oxygen level may portray the induced redox imbalance depicted in Fig. 1, as its accumulation have been implicated in the generation of $O_2^{\cdot -}$ (Patel, 2016). Restoration of these elements to levels indistinguishable from the normal control in the treatment groups, further indicates the neuroprotective effect of *C. nitida* in T2D-brain injury.

The increased expression of Nrf2 in the Purkinje and granular layers in the cerebellar cortices of the untreated diabetic rats (Fig. 8A and B) is in agreement with reports on exacerbated expressions on the onset of T2D (Wrighten et al., 2009; Zucker et al., 2014). Increased expression of Nrf2 has been reported to indicate its translocation into the cell nucleus (Unoki et al., 2018). This translocation has been linked to activation of the antioxidant/electrophile response elements (AREs/EpREs) thereby regulating the cell's defense system (Itoh et al., 1997; Unoki et al., 2018). In the present study, the increased expression of Nrf2 (Fig. 8B) can be correlated with the underlying oxidative stress in the diabetic brain (Fig. 1), which correlates with previous reports on the suppression of the antioxidant system on activation of Nrf2 (He et al., 2012; Miao et al., 2012). These, however, contradict some reports on the beneficial roles of increased Nrf2 expression in the recruitment of the endogenous antioxidants system (Dieter, 2014; Ma, 2013). The attenuated expression in the cerebellar cortex of the treatment groups, may therefore insinuates a suppressive effect of *C. nitida* on hyperglycemia-induced Nrf2 expression in diabetic cerebellum.

6. Conclusion

Taken together, infusion of *C. nitida* confers a neuroprotective effect against hyperglycemia-induced oxidative brain damage in T2D. *C. nitida* attenuates oxidative stress, proinflammation, Nrf2 expression and inhibits the activities of acetylcholinesterase and ATPase, while concomitantly preserving the integrity of the cerebellar cortex. Future studies could be profitably directed at assessing the efficacy of *C. nitida* in combating neurodegenerative diseases.

Conflicts of interest

The authors report no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.03.044.

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