Neuroprotective mechanism of *Vernonia amygdalina* in a rat model of neurodegenerative diseases

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**ABSTRACT**

The global upsurge in the prevalence of neurodegenerative diseases in recent years has been associated with increase in toxic chemical exposure and release into the biosystem, having over 46.8 million people suffer dementia worldwide. This study focused on elucidating the neuroprotective mechanism of methanol leaf extract of *Vernonia amygdalina* (MLVA) in nitrobenzene-induced neurodegenerative disease in rats. Thirty aged male rats were sorted into five groups of six rats each. Group A received distilled water while 100 mg/kg bw of nitrobenzene was orally administered to groups B to E to induce neurodegeneration. Group B (disease control) was untreated, while Group C and D were treated with oral administration of 200 and 400 mg/kg bw of MLVA respectively and group E with vitamin E for 14 days. Locomotor behaviour was analysed using video-tracking software while the midbrain, cerebral and cerebellum of the rats were processed for biochemical analyses.

Results showed that treatment of nitrobenzene-induced neurodegenerative rats with MLVA significantly (p < 0.05) increase dopamine, GSH, antioxidant enzymes levels; and decrease acetylcholinesterase activity, bio-markers of inflammatory and oxidative stress level. Also, MLVA enhanced neurobehavioural and locomotor activities in all markers assessed. Taken together, neuroprotective mechanisms of MLVA can be linked to its antioxidant, acetylcholinesterase suppression, lipid peroxidation inhibition, anti-inflammatory and neuro-behavioural restoring abilities.

**1. Introduction**

The occurrence of neurodegenerative diseases (NDs) is progressively increasing around the globe with more than 46.8 million people suffering from different kinds of the diseases. It is estimated that more than 10 million individuals with NDs will be domiciled in the top 10 most populous nation in the world including Nigeria by 2030 [1–3]. Some of the common risk factors of NDs includes exposure to environmental pollutants, oxidative stress, aging, head trauma, and protein dysfunction. Parkinson’s disease (PD) is of two types: sporadic PD which is more than 90 % cases and familial PD. More than 20 genes have been implicated in parkinsonism [63]. Mutation in UCHL1 (ubiquitin C-terminal hydrolase 1), Parkin, DJ-1 or PINK1 (PTEN induced putative kinase 1) has been reported to cause autosomal recessive while autosomal dominant is caused by genetic mutation in *Vernonia amygdalina* (MLVA) in nitrobenzene-induced neurodegenerative disease in rats. Thirty aged male rats were sorted into five groups of six rats each. Group A received distilled water while 100 mg/kg bw of nitrobenzene was orally administered to groups B to E to induce neurodegeneration. Group B (disease control) was untreated, while Group C and D were treated with oral administration of 200 and 400 mg/kg bw of MLVA respectively and group E with vitamin E for 14 days. Locomotor behaviour was analysed using video-tracking software while the midbrain, cerebral and cerebellum of the rats were processed for biochemical analyses.

Results showed that treatment of nitrobenzene-induced neurodegenerative rats with MLVA significantly (p < 0.05) increase dopamine, GSH, antioxidant enzymes levels; and decrease acetylcholinesterase activity, bio-markers of inflammatory and oxidative stress level. Also, MLVA enhanced neurobehavioural and locomotor activities in all markers assessed. Taken together, neuroprotective mechanisms of MLVA can be linked to its antioxidant, acetylcholinesterase suppression, lipid peroxidation inhibition, anti-inflammatory and neuro-behavioural restoring abilities.

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not yet fully elucidated whether one or more of these diseases mediators is responsible for initiating the disease. Building upon this, medicinal plants, their active components and extracts with multiple pharmacological mechanisms such as antioxidant, anti-apoptotic, and anti-inflammation are strongly recommended as potential neuro-protective agents.

In this study, nitrobenzene was used to induced neurodegeneration in rats. It is a toxic industrial and environmental chemical which was first synthesized by treating benzene with fuming nitric acid in 1834 and was produced for commercial purpose in England in 1856. Since then, it has been produced in very large quantity across the globe due to its relevance in industrial and manufacturing activities [12]. Nitrobenzene and its chemical intermediates are employed in the production of nitrocellulose (pyroxylin), dyestuffs, and in refining lubricating oils [13]. A range of typical symptoms of nitrobenzene toxicosis including weakness, nausea, hyperalgesia, headache, dyspnea, and cyanosis [14]. These symptoms are neuronal related thus suggesting that nitrobenzene may have capacity to interfere with the normal neurological processes.

The current available clinical interventions for the treatment of these NDs are only palliative with associated adverse effects [15], thus there is a need for a new, non-invasive, non-toxic and non-expensive treatment for the diseases. Phytochemicals and natural products of plant origin have been considered to have great beneficial effects to human health and help in the prevention and treatment of many diseases including neurodegenerative disorders [16–18]. Vernonia amygdalina is medicinal plant used traditionally in the treatment and treatment of different diseases [65,66]. The documented phytochemical constituents of various fractions of Vernonia amygdalina includes epivernodalol, sesquiterpene lactones, elemanolide [19], edotides [20], terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones and antraquinone [21], saponins and alkaloids [22]. Documented pharmacological actions of these phytochemical constituents include anti-oxidant properties [23], anti-inflammatory [24].

This study aimed at investigating the protective effects of methanolic leaf extract of Vernonia amygdalina on nitrobenzene-induced neurotoxicity by assessing some locomotor activities and exploratory profiles in experimental rats using standard behavioural protocols and a video-tracking software. Moreover, in order to further understand the neuroprotective mechanisms of the extract in nitrobenzene-induced neurodegeneration in rats, assessment of oxidative stress and inflammatory indices in addition to acetylcholinesterase (AChE), lipid peroxidation and antioxidant enzymes activities were carried out in the midbrain, cerebrum and cerebellum of the treated rats.

2. Materials and methods

2.1. Chemicals and reagents

Vitamin E (Alpha Tocopherol) is a product of Embassy pharmaceuticals, Nigeria. High purity (≤ 99.7 %) Nitrobenzene was obtained from BDH chemical Poole England. Methanol, all other chemicals and reagents are of analytical grade obtained from both Sigma-Aldrich Co. St Louis, Missouri, USA and Analar BDH Limited, Poole, England.

2.2. Vernonia amygdalina

Fresh leaves of Vernonia amygdalina were sourced in a garden at the staff quarters in Kings University, Ode-Omu, Osun state. The leaf has been identified at IFE-Herbarium of Botany Department, Obafemi Awolowo University, Ile-Ife with Voucher number. The Vernonia amygdalina leaves were dried in the Biochemistry laboratory at room temperature and subsequently pulverized. Thereafter, the sample was defatted in n-hexane using Soxhlet apparatus. The methanol extract used for the study was prepared by extracting the defatted Vernonia amygdalina sample for 72 h in 90 % methanol. The resulting mixture was filtered and the filtrate concentrated extract was obtained on water bath. The paste obtained was freeze dried, weighed and reconstituted in water for subsequent studies.

2.3. Phytochemical analysis of Vernonia amygdalina

Phytochemical analysis was carried out on methanol leaf extract of Vernonia amygdalina using standard procedures as described by Harborne [25]. Procedure of Van Buren and Robinson [26] was followed for the quantification of tannin content. The total phenol content was determined according to Singleton et al. [27] while total terpenoids in the extract was determined by method of Ferguson [28]. Alkaloids were quantified by following the procedure of Harborne [25]. Saponins were quantified following the methodology of Obadoni and Ochuko [29], and total flavonoid content was determined according to protocol described by Meda et al. [30].

2.4. Experimental animals

Thirty matured 4–5 months old male Wistar strain albino rats were used in the study. The rats were sourced and raised at the Biochemistry breeding colony of the Biochemistry unit, Department of Chemical Sciences, Kings University, Ode-Omu, Osun state, Nigeria. Animals were kept under ambient standard conditions (25 ± 2 °C and relative humidity of 50 ± 15 %) in stainless steel cages and metabolic wastes were cleaned twice daily. The rats were allowed to acclimatize to these conditions for fourteen days and were exposed to 12 h daylight and darkness cycle, fed with commercially available rat pellet and water ad libitum. The experiment was carried out in accordance with current rules and guidelines that have been established for the care of the laboratory animals [31] with number 2019/001. The rats were grouped into five groups containing six rats each.

- **Group A:** received distilled water daily and serve as the Control.
- **Group B:** received 100 mg/kg Nitrobenzene orally.
- **Group C:** received 100 mg/kg Nitrobenzene and 200 mg/kg Vernonia amygdalina
- **Group D:** received 100 mg/kg Nitrobenzene and 400 mg/kg Vernonia amygdalina
- **Group E:** received 100 mg/kg Nitrobenzene and 400 mg/kg Vitamin E

Treatments were administered to the rats orally for 14 consecutive days.

2.5. Preparation of brain homogenates

The brains were immediately excised and blotted to remove blood stains. They were cleansed and rinsed in 1.15 % KCl on ice to remove haemoglobin, then weighed. They were immediately sectioned into cerebrum, cerebellum, and midbrain then homogenized in four volumes of the homogenizing buffer (10 mM potassium phosphate buffer, pH 7.4) using a Teflon homogenizer. The homogenates were centrifuged at 12,500 g for 15 min in a cold centrifuge (4 °C) to obtain the post mitochondrial fractions which collected and used for biochemical analyses.

2.6. Measurement of biochemical markers

Acetylcholinesterase (AChE) activities in the three regions of the brain were evaluated using the protocol documented by Ellman et al. [32]. The protein content of the homogenates was determined using BSA as a standard in the protocol described by Lowry et al. [33]. Nitric oxide (NO) level was assessed by procedure reported by Green et al. [34]. The concentration of dopamine was evaluated using procedure described by Guo et al. [35]. Myeloperoxidase (MPO) activity in the homogenate was quantified following the method of Granell et al. [36]. Lipid peroxidation was evaluated by monitoring the level of MDA using procedure reported by Varshney and Kale [37]. Superoxide dismutase (SOD)
activity was evaluated following the inhibition of adrenaline auto-oxidation in a basic milieu as described by Misra and Fridovich. [38]. The reduced GSH content in the brain samples was determined using the protocol reported by Buettner et al. [39]. Hydrogen peroxide generation was assayed oxidation of ferrous ions and sorbitol colour amplification system using the method of Wolff, [40]. Catalase (CAT) activity was determined following the protocol documented by Clairborne [41] using hydrogen peroxide (H₂O₂) as a substrate.

2.7. Neurobehavioural assessment

Behavioural assessments to evaluate the motor, exploratory, locomotory, and neuromuscular changes associated with the treatments were conducted 24 h after the last dosage was administered. Open field assessment was used to determine the total distance travelled, total time spent, body angle turn, and average speed as analysed by video tracking software (EthoWatcher). Forelimb grip test, and negative geotaxis using the methods documented by Farombi et al. [16].

2.8. Histological examination

The brains sections were fixed in 10 % formalin and embedded in paraffin wax. Thin sections (7–9 mm thickness) of the liver and kidney tissues were cut and dewaxed in xylene, hydrated in decreasing percentage of alcohol and stained with hematoxylin and eosin (H& E). They were differentiated in 90 % alcohol and cleared in xylene. These stained sections were observed under the microscope for histopathological analysis.

2.9. Statistical analysis

Results obtained were expressed as mean ± standard error of mean (mean ± SD) and analysed using one-way analysis of variance (ANOVA) with the aid of SPSS 22.0 computer software package (SPSS Inc; Chicago, U.S.A) to compare the experimental groups followed by Bonferroni’s post-hoc test. Values at P < 0.05 were considered significant.

3. Results

3.1. Phytochemical constituent of methanol leaf extract of Vernonia amygdalina (MLVA)

The phytochemical screening results revealed the presence of anthraquinone, saponins, alkaloids, flavonoids, lignans, xanthones, cardiac glycosides, terpenes, steroids, coumarins, phenols, tannins and terpenoids as constituent phytochemicals of methanol leaf extract of Vernonia amygdalina. However, as depicted in Table 1, the total flavonoid content of the extract is 108.25 mg/g Catechin equivalent, the total phenol content is 189.52 mg/g Gallic acid equivalent, the tannin content is 146.33 mg/g Gallic acid equivalent while the alkaloids, saponins and total terpenoids are 11.77, 7.52 and 10.71 % yield per gram respectively.

3.2. Effects of MLVA on growth rate, brain weight and body weight of nitrobenzene-induced neurodegenerative rats

Table 2 shows that there is a significant (P < 0.05) decrease in the total body weight gained and brain weights of the rats treated with nitrobenzene alone. The decrease in growth rate of the experimental animals (Fig. 1) also corroborates the observed decline in the total body weight gained and protein content (Fig. 11) of the rats administered with nitrobenzene only. Treatment of the rats with either MLVA or Vitamin E reverse these decrease and enrich the rats’ growth beyond those in the control group.

3.3. MLVA enhanced behavioural, exploratory and locomotor activities of nitrobenzene-induced neurodegenerative rats

Table 3 shows the results of behavioural analyses of the general locomotor activities, negative geotaxis, forelimb grip, time of grooming and incidence of faecal pellets in nitrobenzene-induced neurodegenerative rats. Rats treated with nitrobenzene alone demonstrated significant (P < 0.05) decrease in the total distance travelled, average speed, total time mobile, and turn angle in comparison with control. However, treatment of the rats with either MLVA or Vitamin E showed significant improvement in the motor and locomotor activities evidenced by marked increase in the average speed, total distance travelled, total time mobile and turn angle when compared with untreated neurodegenerative rats. Moreover, Rats treated with nitrobenzene alone exhibited a significant lesser time in forelimb grip test and marked decrease in time of grooming and incidence of faecal pellets than the control. Treatment of the rats with either MLVA or Vitamin E showed marked increase in rat activities on the forelimb grip, time of grooming and faecal pellets when compared with the control.

3.4. MLVA repressed inflammatory activity in brain sections of nitrobenzene-induced neurodegenerative rats

The effects of MLVA on neuro-inflammation in mid brain, cerebrum and cerebellum of the experimental rats was evaluated by measuring MPO activities and NO concentration level. Rats administered nitrobenzene alone demonstrated a marked increase in NO level and MPO activities in all the brain sections when compared with the control (Figs. 2 and 3). However, treatment with of 200 and 400 mg/kg of MLVA or Vitamin E significantly attenuated both NO and MPO levels in the midbrain, cerebrum and cerebellum when compared with control.

3.5. Effects of MLVA on acetylcholinesterase activity and dopamine level in nitrobenzene-induced neurodegenerative rats

Figs. 4 and 5 showed the ameliorating potentials of MLVA on the AChE activity and dopamine level in the three brain regions of the experimental rats. Exposure of rats to nitrobenzene alone caused significant (p < 0.05) decrease in dopamine concentration and elevation in

### Table 1

| S/N | Phytochemical constituents of methanol leaf extract of Vernonia amygdalina (mg/g) | Amount |
|-----|-------------------------------------------------------------------------------------|--------|
| 1   | Total flavonoid (Catechin eq.)                                                      | 108.25 ± 12.73 |
| 2   | Total phenol (Gallic acid eq.)                                                      | 189.52 ± 6.11 |
| 3   | Tannin content (Gallic acid eq.)                                                    | 146.33 ± 8.64 |
| 4   | Alkaloids (Percentage (%) yield per gram)                                           | 11.77 ± 1.85 |
| 5   | Saponins (% yield per gram)                                                        | 7.52 ± 0.96 |
| 6   | Total terpenoids (% yield per gram)                                                 | 10.71 ± 0.55 |

Data are given as mean ± SD of rats per group. n = 6. MLVA: methanolic leaf extract of Vernonia amygdalina. a: Values differ significantly from group A (control) (P < 0.05). b: Values differ significantly from group B (nitrobenzene [NB] only); group C (NB + 200 MLVA), group D (NB + 400 MLVA); group E (NB + 400 MLVA).
Fig. 1. Effects of MLVA on growth rate of rats treated with nitrobenzene. Data are given as mean ± SD of rats per group. n = 6. MLVA: methanolic leaf extract of Vernonia amygdalina. Group A (control), group B (nitrobenzene[NB] only); group C (NB + 200 MLVA), group D (NB + 400 MLVA); group E (NB + 400 MLVA).

Table 3

| Effect                               | Group A       | Group B       | Group C       | Group D       | Group E       |
|--------------------------------------|---------------|---------------|---------------|---------------|---------------|
| Total distance travelled (cm)        | 93.65 ± 0.13  | 50.43 ± 0.47  | 85.33 ± 2.80  | 92.87 ± 3.11  | 89.25 ± 6.01  |
| Total time (Sec)                     | 306.10 ± 8.32 | 253.90 ± 12.40| 315.90 ± 17.64| 330.18 ± 9.10 | 323.05 ± 7.50 |
| Body angle turn (°)                  | 6.03 ± 0.17   | 11.05 ± 0.52  | 12.79 ± 0.72  | 9.29 ± 0.75   | 10.16 ± 0.12  |
| Average speed (cm/sec)               | 0.31 ± 0.06   | 0.20 ± 0.03   | 0.27 ± 0.04   | 0.28 ± 0.04   | 0.28 ± 0.04   |
| Grooming (Sec)                       | 76.50 ± 7.85  | 10.65 ± 4.13  | 85.40 ± 13.02 | 91.35 ± 17.48 | 88.10 ± 12.64 |
| Negative geotaxis (Sec)              | 1.40 ± 0.35   | 1.90 ± 0.45   | 1.50 ± 0.60   | 1.70 ± 0.60   | 1.70 ± 0.60   |
| Fecal pellets (frequency)            | 1.10 ± 0.35   | 1.20 ± 0.45   | 1.20 ± 0.50   | 1.50 ± 0.50   | 1.40 ± 0.50   |
| Fore-limb grip (Sec)                 | 47.50 ± 5.61  | 13.02 ± 12.60 | 17.48 ± 12.64 | 12.64 ± 10.42 | 12.48 ± 10.42 |

Data are given as mean ± SD of rats per group. MLVA: methanolic leaf extract of Vernonia amygdalina, a: Values differ significantly from group A (control) (P < 0.05). b: Values differ significantly from group B (nitrobenzene[NB] only); group C (NB + 200 MLVA), group D (NB + 400 MLVA); group E (NB + 400 MLVA).

Fig. 2. Effect of MLVA on nitric oxide level in brain regions of nitrobenzene-induced neurodegenerative rats. Data are given as mean ± SD of rats per group. MLVA: methanolic leaf extract of Vernonia amygdalina, a: Values differ significantly from group A (control) (P < 0.05). b: Values differ significantly from group B (nitrobenzene[NB] only); group C (NB + 200 MLVA), group D (NB + 400 MLVA); group E (NB + 400 MLVA).

Fig. 3. Effect of MLVA on myeloperoxidase (MPO) activities level in brain regions of nitrobenzene-induced neurodegenerative rats. Data are given as mean ± SD of rats per group. MLVA: methanolic leaf extract of Vernonia amygdalina, a: Values differ significantly from group A (control) (P < 0.05). b: Values differ significantly from group B (nitrobenzene[NB] only); group C (NB + 200 MLVA), group D (NB + 400 MLVA); group E (NB + 400 MLVA).

Fig. 4. Effect of MLVA on acetylcholinesterase (AChE) activities level in brain regions of nitrobenzene-induced neurodegenerative rats. Data are given as mean ± SD of rats per group. MLVA: methanolic leaf extract of Vernonia amygdalina, a: Values differ significantly from group A (control) (P < 0.05). b: Values differ significantly from group B (nitrobenzene[NB] only); group C (NB + 200 MLVA), group D (NB + 400 MLVA); group E (NB + 400 MLVA).

Fig. 5. Effect of MLVA on dopamine level in brain regions of nitrobenzene-induced neurodegenerative rats. Data are given as mean ± SD of rats per group. MLVA: methanolic leaf extract of Vernonia amygdalina, a: Values differ significantly from group A (control) (P < 0.05). b: Values differ significantly from group B (nitrobenzene[NB] only); group C (NB + 200 MLVA), group D (NB + 400 MLVA); group E (NB + 400 MLVA).
AChE activity in the midbrain, cerebrum and cerebellum of the rats. However, treatment with either MLVA and vitamin E significantly (p < 0.05) reversed the AChE activity when compared with the control and enhance dopamine concentrations in all regions of the brain examined.

3.6. MLVA improved glutathione level and antioxidant enzymes activities in brain sections of nitrobenzene-induced neurodegenerative rats

Figs. 6–8 shows the glutathione level and antioxidant activities of CAT and SOD in the midbrain, cerebrum and cerebellum of experimental rats. Administration of rat with nitrobenzene alone showed caused a significant decrease (p < 0.05) in GSH level and decline in activities of CAT and SOD when compared with the control. However, treatment with 200 and 400 mg/kg of MLVA or Vitamin E significantly increased the GSH level and enhance all the enzyme.

3.7. MLVA suppressed lipid peroxidation and hydrogen peroxide generation in brain sections of nitrobenzene-induced neurodegenerative rats

Figs. 9 and 10 showed the results of neuronal oxidative stress biomarkers carried out in the midbrain, cerebrum and cerebellum of experimental rats. There was a marked increase in MDA level (an index of lipid peroxidation) and H$_2$O$_2$ generation in the midbrain, cerebrum and cerebellum of rats administered nitrobenzene only when compared with the control group. Upon treatment with 200 and 400 mg/kg of MLVA or Vitamin E, there was a significant decrease (p < 0.05) in both MDA and H$_2$O$_2$ generation levels in the regions of the brain assessed.

3.8. Reversal effects of MLVA on histological alterations in brain sections of nitrobenzene-induced neurodegenerative rats

Figs. 12–15 show the histological alterations seen with the light microscope in the hippocampus, midbrain, cerebrum and cerebellum sections of brains of the experimental rats. The cytoarchitecture and morphology of hippocampus, midbrain, cerebrum and cerebellum of rats from control group appeared normal. However, obvious pathological lesions were observed in the hippocampus, midbrain, cerebrum and cerebellum sections of nitrobenzene group. In cerebellar cortex (Fig. 12), group B show mild degenerative characterized with loss of cells from the purkinje cell layer and gradual loss of cytoplasmic contents, fragmented granule cell layer as well loss of major parts of the neuropil (red arrows). Group E showed a mild similar appearance with A and D group (yellow arrow). In cerebral prefrontal cortex (Fig. 13), Group B treatment caused some observable degenerative changes in the

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**Fig. 6.** Effect of MLVA on reduced glutathione (GSH) content in brain regions of nitrobenzene-induced neurodegenerative rats.

Data are given as mean ± SD of rats per group. MLVA: methanolic leaf extract of Vernonia amygdalina, a: Values differ significantly from group A (control) (P < 0.05). b: Values differ significantly from group B (nitrobenzene[NB] only); group C (NB + 200 MLVA), group D (NB + 400 MLVA); group E (NB + 400 MLVA).

**Fig. 7.** Effect of MLVA on catalase (CAT) activities in brain regions of nitrobenzene-induced neurodegenerative rats.

Data are given as mean ± SD of rats per group. MLVA: methanolic leaf extract of Vernonia amygdalina, a: Values differ significantly from group A (control) (P < 0.05). b: Values differ significantly from group B (nitrobenzene[NB] only); group C (NB + 200 MLVA), group D (NB + 400 MLVA); group E (NB + 400 MLVA).

**Fig. 8.** Effect of MLVA on superoxide dismutase (SOD) activities in brain regions of nitrobenzene-induced neurodegenerative rats.

Data are given as mean ± SD of rats per group. MLVA: methanolic leaf extract of Vernonia amygdalina, a: Values differ significantly from group A (control) (P < 0.05). b: Values differ significantly from group B (nitrobenzene[NB] only); group C (NB + 200 MLVA), group D (NB + 400 MLVA); group E (NB + 400 MLVA).

**Fig. 9.** Effect of MLVA on lipid peroxidation (MDA) levels in brain regions of nitrobenzene-induced neurodegenerative rats.

Data are given as mean ± SD of rats per group. MLVA: methanolic leaf extract of Vernonia amygdalina, a: Values differ significantly from group A (control) (P < 0.05). b: Values differ significantly from group B (nitrobenzene[NB] only); group C (NB + 200 MLVA), group D (NB + 400 MLVA); group E (NB + 400 MLVA).
cortex that was characterized by clustered pyknotic pyramidal neurons that appear with fragmented cytoplasm and condensed nuclei within soma (red arrow). Perineurial spaces can be seen surrounding degenerating neurons (red arrows) Axons and dendrites are sharply appreciable around neurons in this group. Also, nuclear content and cytoplasmic materials are less appreciable. In hippocampus (Fig. 14), group B treatment on the other hand, induced degenerative changes in the hippocampus and was characterised by fragmented pyramidal and granule cell layer. Although group C and E showed slight degenerative changes in line with the morphologic appearance of groups B; their cortical layers are better structured and delineated like group A and D. In midbrain (Fig. 15), Plates with marked morphological alteration is indicated by red arrow while yellow arrow indicates plate with mild morphological alteration. Plate with no observable alteration is indicated by black arrow.

4. Discussion

The upsurge in the prevalence of neurotoxicity induced by exposure to harmful chemicals is a major global health challenge [67,68]. Several characteristic factors such as dopamine depletion, increase in oxidative stress, acetylcholinesterase (AChE) activity, genetic mutations, aggregation of toxic misfolded proteins, proteosomal dysfunction, decrease in acetylcholine (ACh), brain-derived neurotrophic factor (BDNF) in the brain are involved in aetiology of NDs [6,42]. Preclinical trials on drug candidates have revealed several mechanisms underlying the observed neuroprotective effects of medicinal plants and their extracts, such as anti-inflammatory, anti-apoptotic, antioxidant, and specific neurotrophic mechanisms [43]. Molecular elucidation of these mechanisms will enhance discovery of novel neuroprotective agents for NDs. Thus, prophylactic approach using phytochemical may offer a significant clinical intervention in these pathological conditions.

This present study demonstrated that methanol leaf extract of Vernonia amygdalina has remarkable protective effects in the treatment of biochemical and neurobehavioural deficits in rats exposed to neurotoxicants. Results from this study revealed that there was a drastic reduction in brain weight of rats administered with nitrobenzene alone with concomitant decrease in the total body weight gained. Several experimental reports have demonstrated chemical toxicities lead to significant brain and body weight loss in rats if left untreated over time [16,44,45]. The observed reduction in the brain and body weight can be caused by depletion of nutrients, alteration of metabolic activities or pathways and inhibition in protein synthesis [44]. However, administration of 100 and 200 mg/kg body weight of methanol leaf extract of Vernonia amygdalina or vitamin E significantly increase both the brain and body weight of the rat to normal. This suggested the ability of the extract to improve metabolic activities and enhance protein synthesis in the brain.

In this study, experimental animals exposed to nitrobenzene alone demonstrated notable locomotor alterations as shown by decrease in speed, total mobility time and total distance covered. Furthermore, there is significant decline in the forelimb grip, body rotation and turn angle which are essential psychomotor coordination tools to assess neurodegenerative disorders [46,47]. The observed decrease in these locomotor markers implies dysfunction in the cellular signalling between nervous system and muscular junctions. Treatment with methanol leaf extract of Vernonia amygdalina or vitamin E significantly increase all these markers indicating their abilities to reverse chemically mediated psychomotor dysfunctions in rats. Grooming in rats is a standard neurobehavioural index to evaluate alterations in behavioural patterns and psychomotor activities in rats [48]. Therefore, the role of methanol leaf extract of Vernonia amygdalina in reversing nitrobenzene-induced decrease in grooming time further potentiate beneficial effects of the extract against psychomotor deficiency in rats.

Similarly, rats administered nitrobenzene only exhibited marked neuro-behavioural deficits as revealed by decrease in the exploratory activities and increased in frequency of faecal pellets. Exploratory activities and frequency of faecal pellets are parameters used to determined anxiogenic-like phenotype and behavioural imbalance in rats [49]. Treatment with 100 and 200 mg/kg body weight of methanol leaf extract of Vernonia amygdalina or vitamin E significantly influence the behavioural activities of the rats by enhancing the exploratory events. This confirm the neuro-behavioural benefits of the extract.

In this study, exposure of rats to nitrobenzene induced significant increase in the level of malondialdehyde (MDA) as marker of lipid peroxidation; and marked increase in NO and MPO as markers of inflammatory respectively in the three regions (cerebrum, cerebellum, and mid brain) of the brain. Neuronal cells are more vulnerable to oxidative stress and inflammation, thus, requires efficient steady supply of potent antioxidants to neutralize the lipid peroxidation chain reaction, which induces free radical damages. Treatment with methanol leaf extract of Vernonia amygdalina or vitamin E significantly reverse the increase thus, inhibiting the lipid peroxidation chain reaction and neuro-inflammation in the three regions of the brain examined. These findings corroborate the report of Layer and Gomez-pinilla [50] that exogenous antioxidants obtained from plants or other natural products influence brain function and health.

Acetylcholine is an essential neurotransmitter in the cholinergic system which play a major role in both central nervous system and...
peripheral nervous system functions including learning, memory, movement, control and modulation of cerebral blood flow [51–53] and thus very important in neurological functions. Acetylcholine level is controlled by Acetylcholinesterase (AchE), an enzyme that hydrolyse the neurotransmitter. Alteration in the activities of AchE may lead to undesirable effect on cholinergic transmission as well as progressive cognitive impairment [54]. Administration of nitrobenzene to rats cause drastic increase in the level of acetylcholinesterase activity in the three regions (cerebrum, cerebellum, and the mid brain) of the brain examined. Treatment with methanol leaf extract of Vernonia amygdalina or vitamin E significantly attenuate this effect by decreasing greatly the activity of this enzyme in the three regions of the brain, especially in the striatum which contribute greatly in the neurodegenerative disorder known as Parkinson’s disease. This established the ability of the extract to increase acetylcholine level in the synaptic cleft and influence cholinergic neurotransmission.

Equally, the mechanism of action of some medicinal plants used in the management of Alzheimer’s disease and memory deficit disorders has been linked to their modulatory effects on acetylcholinesterase activity which enhances learning and memory [55]. This cholinergic enzyme is highly implicated in cognitive dysfunction and pathogenesis of Alzheimer’s disease, because clinical studies have shown that the sufferers of cognitive dysfunction, exhibited some behavioural improvements when cholinesterase inhibitor therapy commenced [56]. Thus, the ability of Vernonia amygdalina to increase the bioavailability of acetylcholine by inhibiting the activities of acetylcholinesterase suggested that the extract has memory enhancing effect.

Dopamine also perform critical function in motor functions [57]. The depletion and consequent energy dysfunction of the mitochondria cascades affects the dopaminergic neurons, which requires greater energy

Fig. 12. Representative histological sections of panoramic views of cerebellar cortex of experimental rats. H & E stain (x40, x100 and x400 magnification respectively). Molecular layer (M), purkinje cell layer (P), granule cell layer (G) and the medullary layer of white matter (W). Group A (control), group B (nitrobenzene[NB] only); group C (NB + 200 MLVA), group D (NB + 400 MLVA); group E (NB + 400 MLVA).

Fig. 13. Representative histological sections of panoramic views of cerebral prefrontal cortex of experimental rats. H & E stain (x40, x100 and x400 magnification respectively). The molecular layer (I), External granular layer (II), External pyramidal layer (III), Internal granular layer (IV), Internal pyramidal layer (V) and the multiform layer (VI) are demonstrated across study groups A–E (arrow head direction). Group A (control), group B (nitrobenzene[NB] only); group C (NB + 200 MLVA), group D (NB + 400 MLVA); group E (NB + 400 MLVA).
Fig. 14. Representative histological sections of panoramic views of hippocampus of experimental rats. H & E stain (x40, x100 and x400 magnification respectively). The Dentate gyrus (DG) composed of granule cells, Cornu amonos (CA 1-3) containing pyramidal cells, are well demonstrated across the study groups. Group A (control), group B (nitrobenzene [NB] only); group C (NB + 200 MLVA), group D (NB + 400 MLVA); group E (NB + 400 MLVA).

Fig. 15. Representative histological sections of experimental rats’ midbrain showing regions of magnocellular red nucleus of nerve cells with large prominent pale indented nuclei, prominent nucleoli, fusiform and pyramidal nerve cells are seen. Cell processes are seen as well as some neuroglia. H & E stain (x400 magnification). Group A (control), group B (nitrobenzene [NB] only); group C (NB + 200 MLVA), group D (NB + 400 MLVA); group E (NB + 400 MLVA).
for efficient function. In this study, nitrobenzene greatly reduced dopamine activity in the striatum, cerebellum and the mid brain. The severity of the clinical manifestations of parkinsonism (motor disturbance) and dementia is associated largely with the degeneration of dopaminergic neurons resulting in reduction of brain dopamine content [16]. Treatment with methanol leaf extract of Vernonia amygdalina or vitamin E significantly increase the level of dopamine activity especially in the striatum. Certain plant nutrients serve as main precursors in the synthesis of neurotransmitters such as epinephrine, acetylcholine, serotonin, and dopamine [59].

One of the beneficial effects of phytochemicals is their ability to contribute immensely to the general antioxidant defense system in various organs in the body including the brain, thereby influencing brain function, cognitive ability, and preventing oxidative stress [16]. In this study, exposure of rats to Nitrobenzene cause oxidative stress with significant decrease in GSH, CAT, and SOD. The reduction in the activities of these antioxidant enzymes is well-known to provide evidence about oxidative damage as a result of accumulation of ROS including H2O2 which eventually leads to lipid peroxidation [60, 61, 69, 70]. However, treatment with methanol leaf extract of Vernonia amygdalina or vitamin E significantly increase the activity levels of these enzymes. This result agrees with previous report that endogenous antioxidants enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX), glutathione S-transferase (GST) play significant role in inhibiting neurodegeneration in the brain [62].

Histological study shows the cytoarchitecture of three regions of the brain. There are marked alterations in morphological characteristics in the cerebrum, midbrain and cerebellum of the group of rats administered with nitrobenzene only. The control group and group treated with Vernonia amygdalina and vitamin E showed no changes or alterations in their morphological characteristics. The mid brain and cerebellum of the toxicant group showed observable gradual loss of cytoplasmic contents and degenerative changes like clustered pyknotic (irreversible condensation of the chromatin) pyramidal neurons was seen at the cerebral cortex. Apoptosis (programmed cell death) is characterized by pyknosis. Therefore, the feature exhibited in the cerebral cortex can be explained as altered rate of apoptosis, this can lead to excessive presence and accumulation of dead neuronal cells. This histological finding corroborates the biochemical results.

5. Conclusion

Data from this study further potentiate the beneficial effects of methanol leaf extract of Vernonia amygdalina in neurodegenerative disorders. The results obtained demonstrated with convincing evidence that behavioural deficit associated with administration of nitrobenzene in rats were markedly attenuated by methanol leaf extract of Vernonia amygdalina. In addition, the extract mitigated the molecular processes and pathological features associated with neurodegenerative disorders in the cerebrum, cerebellum, and midbrain via mechanisms related to its antioxidant, acetylcholinesterase suppression, lipid peroxidation inhibition, anti-inflammatory and neurobehavioural restoring properties. Thus, our data suggest the clinical significance of methanol leaf extract of Vernonia amygdalina in the prevention and management of neurodegenerative disorders with behavioural impairment.

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CRedit authorship contribution statement

John O. Oladele: Conceptualization, Methodology, Data curation, Writing - original draft, Funding acquisition. Oyedotun M. Oyeleke: Supervision, Project administration, Writing - original draft, Funding acquisition. Oluwaseun T. Oladele: Methodology, Data curation, Writing - original draft, Funding acquisition. Monisola Olaniyi: Methodology, Data curation, Writing - original draft, Funding acquisition.

Declaration of Competing Interest

The authors report no declarations of interest.

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References

[1] E.R. Dorsey, R. Constantinescu, J.P. Thompson, K.L.M. Biglan, R.G. Holloway, K. E. Al Kiehurst, Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030, Neurology 68 (2007) 384–386.
[2] N.U. Okubadejo, O.O. Ojo, O.O. Gbaja-Amosu. Clinical profile of parkinsonism and parkinson’s disease in Lagos, South Western Nigeria, BMC Neurol. 10 (2010) 1.
[3] Parkinson Disease Foundation (PDF), Statistics on parkinson’s [WWW Document]. URL, 2016, http://www.pdpf.org/syptoms-statistics.
[4] C. Xav, R. El Khouby, W. Wang, et al., LRRK2-mediated neurodegeneration and dysfunction of dopaminergic neurons in a Caenorhabditis elegans model of Parkinson’s disease, Neurobiol. Dis. 40 (2010) 73–81, https://doi.org/10.1016/j. nbd.2010.04.002.
[5] A.H. Hellerich, P.J. McLean, J.H. Weinhaupt, K.M. Danzer, Commentary: alpha-synuclein interacts with SOD1 and promotes its oligomerization, J. Neuro. Neuron. 1 (2016) 28–30.
[6] A.R. Winslow, C.W. Chen, S. Corrochano, et al., a-Synuclein impair macroautophagy: implications for Parkinson’s disease, J. Cell Biol. 190 (2010) 1023–1037.
[7] R.S. Wilson, E. Segawa, P.A. Boyle, et al., Psychology and aging the natural history of cognitive decline in Alzheimer’s disease, Nat. Hist. Cognit. Decline Alzheimer’s Dis. 27 (2012), https://doi.org/10.1007/s12128-012-9588-z.
[8] J. Gaugler, B. James, T. Johnson, et al., 2016 Alzheimer’s disease facts and figures, Alzheimer’s Dementia 12 (2016) 459–509, https://doi.org/10.1017/j. jad.2016.03.001.
[9] K. Mi, G. Johnson, The role of Tau phosphorylation in the pathogenesis of Alzheimers disease, Curr. Alzheimer Res. 3 (2006) 449–463, https://doi.org/10.2174/156720506779025279.
[10] M.P. Murphy, H.L. Iii, AD and beta amyloid peptide, J. Alzheimers Dis. 19 (2010) 1–47, https://doi.org/10.3231/JAD.2010.1221.Alzheimer.
[11] T. Pringsheim, N. Jette, A. Frolikis, T.D.L. Steeves, The prevalence of Parkinson’s disease: a systemic review and meta-analysis, Mov. Disord. 29 (2014) 1583–1590.
[12] IPCS (International Programme on Chemical Safety), Nitrobenzene, Environmental Health Criteria, vol. 230, World Health Organization, Geneva, Switzerland, 2003. Available online at, http://www.inchem.org/documents/ehc/ehc/ehc230.htm.
[13] RAR, Part I, Environment European Union Risk Assessment Report Nitrobenzene, 2007, pp. 2–106. CAS# 98-95-3, Final Risk Assessment Report, Environmental Part (RAR, 2007), http://eisj.jrc.ec.europa.eu/.
[14] ECHA (European Chemical Agency), Background Document to RAC Opinion on Nitrobenzene, 2012. ECHA-RAC-CHI-O-000002350-87-01-A1.
[15] S. Costello, M. Cockburn, J. Bronstein, X. Zhang, B. Ritz, Parkinson’s disease and residential exposure to maneb and paraquat from agricultural applications in the central valley of California, Am. J. Epidemiol. 169 (2009) 919–926.
[16] E.O. Fanombi, L.O. Awogbindin, T.H. Fanombi, J.O. Oladele, E.R. Izomoh, O. B. Aladelokun, O.I. Ezekiel, O.I. Adebambo, V.O. Abah, Neuroprotective role of Ficus exasperata leaf attenuates arsenate-mediated hepatic and renal oxidative stress in rats, Res. J. Health Sci. 5 (2) (2017) 115–123, https://doi.org/10.4314/rjhs.v5i2.7.
[17] O.I. Oyewole, B.F. Olayebi, J.O. Oladele, Antioxidative potential of Ricinus communis leaf extract on cadmium-induced liver and brain toxicity in Rats, UNIOSUN J. Sci. 2 (2017) 84–90.
[18] P. Erasto, D.S. Grierson, A.J. Al方圆, Bioactive sesquiterpenes lactones from the leaves of V. amygdalina, J. Ethnopharmacol. 106 (2006) 117–120, https://doi.org/10.1016/j.jep.2005.12.016.
