The Journal of Anatomical Sciences
Email: journalofanatomicalsciences@gmail.com

J. Anat Sci 15(1)

Neuroprotective Effects of *Buccholzia coriacea* Seed on $AlCl_3$-induced Memory Impairment in Adult Albino Rats

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ABSTRACT

Memory impairment is a neurodegenerative condition that is associated with poor cognition. Aluminium chloride ($AlCl_3$) is a neurotoxic agent that disrupts the structure-function of the brain, leading to impaired cognition and memory. *Buccholzia coriacea* is traditionally reputed to boost memory. This study aimed at testing the aforementioned hypothesis through the use of *B. coriacea* seed and vitamins C and E on $AlCl_3$-induced memory impairment in adult albino rats. Twenty-five female rats were divided into five groups of five rats each. Each group was administered: distilled water (control group), 1 ml/kg BW olive oil, 100 mg/kg BW $AlCl_3$ (negative control), 100 mg/kg BW $AlCl_3$ followed by 100 mg/kg BW vitamins C and 1000 IU/kg BW of vitamin E and the last group received 200 mg/kg BW of *B. coriacea* after 100 mg/kg BW $AlCl_3$ administration for 28 days. $AlCl_3$ administration lead to impaired memory and spatial learning. Treatment with *B. coriacea* improved memory and improved memory retention and modulated brain oxidative stress levels as observed from the depleted level of malondialdehyde and increased levels of the oxidized glutathione peroxidase and superoxide dismutase levels of the hippocampus. In conclusion, our study demonstrates that the use of *B. coriacea* is a promising plant for reducing oxidative stress, neurotoxicity, and for memory enhancement.

Keywords: memory, *Buccholzia coriacea*, hippocampus, oxidative stress

INTRODUCTION

Neurotoxicity occurs when neuronal cells or nervous tissues are exposed to neurotoxins. Neurotoxicity can also be defined as any harmful effect on the structure, function or biochemistry of the nervous system during development or at maturity, elicited by physical or chemical factors[1]. Various chemical substances have been known to elicit neurotoxicity in humans and as experimental tools to produce damage to the nervous system in animals. Exposure to heavy metals (such as lead, mercury, and $AlCl_3$), pesticides, insecticides, radiation treatment, certain food additives, naturally occurring substances as well as chemotherapy can result into neurotoxicity[2]. Some toxins can lead to loss of neurons (neuronopathy), loss of glial cells, damage to neuronal axon (axonopathy) or any other form of gliopathies. Neurotoxicity can lead to neurodegenerative diseases (which is a constellation of disorders characterized by gradual and persistent loss of neuronal function). The etiology of neurodegenerative diseases is multifactorial, involving a byzantine combination of genetic and environmental factors such as aging process, as is the case with Alzheimer's disease.

Aluminium is considered as one of the ubiquitous metals on earth, ranked as the third most abundant after oxygen and silicon. The environmental level of aluminium has increased tremendously[3], thanks to intensive growth in aluminium industries[4]. Common sources of aluminium contaminants include diet[5], accounting to over 90% of the total body aluminium, ambient air, drinking water, cosmetics and medications especially antacids[4,6-9]. Other indirect risk factors for aluminium toxicity include occupational hazards (for people working in aluminium industries) and vaccination (especially in vaccines with

aluminium adjuvants). Aluminium has been reported to be toxic to humans[9] leading to both neurodegenerative and neurodevelopmental disorders.

Exposure to aluminium has been reported to cause neurodegenerative disorders by adversely affecting various biomolecules associated with neurotoxicity[10,11]. Aluminium is also known to catalyze oxidative stress (either during cellular respiration or by inflammatory cells in response to invading pathogens), deposition of amyloid beta (Aβ) oligomers and production of plaques in the hippocampus and cerebral cortex[12]. Hence, AlCl3-induced neurodegeneration in rats is a good model for understanding the neuroprotective effects of phytochemicals. The abundance of aluminium in the environment, food and daily life activities make it almost inevitable for exposure[13,14.]

Researchers have shown that an array of plant-originated phytochemicals have neuroprotective potentials and further identification of such compound may be an effective strategy for decreasing neurodegenerative disorders. For instance, *B. coriacea* seeds have been reported to have hypoglycaemic, anti-microbial and anti-hypertensive effects[15-18]. Furthermore, its bark has been traditionally reputed for the treatment of pleurisy, sinusitis, bronchitis, headache, kidney pains, ophthalmia and nasal congestion. Results of the phyto-constituent studies of *B. coriacea* revealed that the seeds are rich in tannins, saponins, alkaloids and flavonoids [19,20]. Several medicinal plants with neuroprotective properties have been reported to be rich in these phytochemicals [21-23]. Other phyto-constituents that have been reported to have neuroprotective effects are: thymol (a monoterpene phenol that is commonly isolated from medicinal plants), which has been shown to have good neuroprotective effects by enhancing cognitive skills in a model of dementia[24-26] and thymoquinone, the active phytochemical in *Nigella sativa* which has been reported to ameliorate learning dysfunction, increased number of neurons, reduction in plaque formation in the hippocampus and protect pyramidal cells from neurotoxic insults of Aβ[27,28]. Another phytochemical that has been reputed for its neuroprotective property is morin, a pale yellow crystalline polyphenol, readily found in several vegetables and fruits[29]; in addition to having neuroprotective properties, also has antibacterial, antitumoral and anti-inflammatory effects [30]. Furthermore, phytochemicals can exert neuroprotective effects through reactive oxygen species[31]. Although many researchers have investigated the health benefits of *B. coriacea,* its neuroprotective benefits still require comprehensive attention. Knowledge of the neuroprotective effects promises to enhance therapeutic and preventive strategies for neurological disorders such as Parkinson and Alzheimer's diseases.

Vitamin C, also known as L-ascorbic acid, is a water-soluble vitamin that is naturally present in some fruits. It is also available as a dietary supplement but it is easily destroyed by heat and its potency is reduced by prolonged storage[32]. Humans, unlike most animals, are unable to synthesize vitamin C endogenously, so it is an essential dietary component[33,34].

Vitamin E is a generic name for tocopherols and tocotrienols. Tocopherol contains saturated phytol side chains and tocotrienol has 3 double bonds in its side chain [35,36]. The alpha-tocopherol form of vitamin E is an important lipid-soluble antioxidant. In the brain and other tissues, alpha-tocopherol plays a key role in preventing oxidant-induced lipid destruction and is, therefore, vital in maintaining the integrity of cell membranes [35,36]. Accordingly, vitamin E deficiency causes lipid peroxidation in brain tissues. The study aimed at investigating the possible neuroprotective effect of *B. coriacea* following $AlCl_3$-induced neurotoxicity in a rat model.

MATERIALS AND METHODS

Animals and grouping

Female albino rats used in this study (160 – 190 g in weight), 8 – 9 weeks old were purchased from the animal house of the Department of Human Physiology, Bingham University, Karu, Nasarawa State, Nigeria. Rats were kept in plastic cages (5 rats per cage) in a room with ambient temperature of about 28 ± 1 ℃ and had free access to food and water *ad libitum* and 12 hours light-dark cycle with all experimental procedures conducted during light phase. Tail tagging was used to differentiate rats in each group. The study protocol, animal treatment and handling methods were reviewed and approved by Bingham University Committee on Ethics. Further, all experimental procedures were conducted in keeping with the relevant regulations and guidelines for the Care and Use of Laboratory Animals as published by the National Institutes of Health.

Five groups (I – V) of five animals per group were randomly assigned into:

Group I: the rats were the positive control and were administered distilled water for 28 days.

Group II: were orally administered 1 ml/kg BW of olive oil for 28 days.

Group III: were orally administered 100 mg/kg BW of $AlCl_3$ for 28 days and considered as negative control.

Group IV: were orally administered 100 mg/kg BW of $AlCl_3$ for 21 days followed by 200 mg/kg BWof ethanolic extract of *Buccholzia coriacea* for 28 days.

Group V: were orally administered 100 mg/kg BW of $AlCl_3$ for 21 days followed by 100 mg/kg BW and 1000 IU/kg BW of vitamins C and E respectively for 28 days.

Drugs and chemicals

Ascorbic acid tablets (100 mg/tablet) (Med Vitamin C, Dol-Med Laboratories Limited, Lagos, Nigeria) were obtained from a Pharmaceutical store in Nasarawa state, Nigeria. Each tablet was dissolved in 20 ml of distilled water to obtain 100 mg/kg suspension. Vitamin E (1000 IU/capsule) (karma PharmaTech Ltd) was also obtained from a pharmaceutical store in Nasarawa state, Nigeria. Each vitamin E tablet was dissolved in 700 ml of olive oil. Olive oil was obtained from a pharmaceutical store at Mararaba market, Nasarawa state, Nigeria. All chemicals used for this study met analytical grade.

Fresh seeds of *Buccholzia coriacea* were obtained from Lagos State, Southwest Nigeria, and were authenticated at the Department of Botany, University of Lagos, Akoka, Lagos, Nigeria. The Voucher, Capparaceae LUH 8022, specimen was deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Lagos, Akoka, Lagos, Nigeria. The seeds were dried, grated into tiny pieces, and then ground into a rough powder. Cold maceration was used for extraction. With intermittent shaking every two hours, one kilogram of the plant material was extracted in distilled water for 72 hours. Whatman No. 1 filter paper was then used to filter the extract. A freeze dryer was used to lyophilize the filtrate after it had been concentrated using a rotary evaporator (Rota vapor R 210, Büchi, Switzerland) at 40°C. Before use, the yield was calculated and the extract was kept in sample containers in a refrigerator at 4°C.

Neurobehavioural Assessments

Morris Water Maze (MWM)

The MWM was used to study spatial navigation as previously described [37]. Briefly, animals were placed in a pool of circular water tank (180 cm in diameter and 90 cm deep). The water tank was half-filled with pipe-borne water and subsequently coloured opaque with non-toxic tempera paint. The animals were expected to find the hidden platform. The pool was divided into four equal quadrants (east, west, north and south) as starting positions. The escape platform is 10 cm in diameter and 2 cm below the water level at a fixed position in the centre of one of the quadrants. The animals were gently lowered into the water tail-end by supporting it with the hand. The animal is gleefully allowed to search for the escape platform for a maximum of one minute, although it was guided to the target arm if unable to find the escape platform at the end of 60 seconds. Also, each animal was allowed to spend 20 seconds on the target arm to explore its location based on the visual cues. During memory test, each rat was given one minute to spot the escape platform (with 20 seconds to rest on the platform). An error will be recorded in an event the rat entered another arm other than the escape platform. Also, the amount of time it took each rat to locate the escape platform (escape latency) was also recorded in seconds.

Probe trials to test memory

After training was completed, probe trial was conducted in which the escape platform was removed from the pool, and the animal were allowed to swim for one minute to search for the escape platform. Typically, a well-trained rat will swim toward the position of the escape platform repeatedly. The number of crossings through the position of the escape platform was counted and recorded. This spatial bias constitutes evidence for spatial memory.

Tissue preparation and assembly

Blood samples were collected from the retro-orbital venous sinus under light anaesthesia. Subsequently, the animals were euthanized with a high dose of ketamine (100 mg/kg) before sacrifice. Brain tissues were dissected out and placed on a filter paper soaked with normal saline. The filter paper was then placed on a glass plate filled with crushed ice. Both lobes (left and right) of the hippocampus were separated from other parts of the cerebral cortex and placed in Eppendorf tube earlier pre-labeled for easy identification. The tubes were stored in a refrigerator at 4℃ until further analysis. Five samples of brain and blood tissues were collected per group for histopathological studies. The blood tissue was used for biochemical analyses.

Histopathological assessment

The brain tissues of the hippocampus were rinsed with isotonic sodium chloride solution, then homogenized in phosphate buffer (200 µl). The homogenization buffer was prepared by the mixture of two tablets of protease inhibitor and one phosphate buffered saline tablet in 200 ml distilled water. Next, the mixture of homogenized buffer and tissues were centrifuged at 15000xg for 600 seconds at 4°C in order to remove undissolved tissues from the buffer and hence remained insoluble. Consequently, the supernatant

from the preceding step was collected and stored till further analysis.

Colorimetric analysis

The brain tissue homogenates were assessed for oxidative stress biomarkers. Total GSH-Px, SOD and malondialdehyde were measured by commercially available colorimetric assay kits based on kit's manufacturer's protocols. For each assay, the absorbance was measured spectrophotometrically at specified wavelength in each assay kit for the three oxidative stress biomarkers.

Data analysis

Results are expressed as mean ± SE and graphs. Analysis of variance was used to compare means across groups. Where statistically significant difference is observed, this was followed by John Tukey's Honest Significant Difference test for pairwise comparisons. All statistical analyses were performed using Statistical Product and Service Solutions (IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp). A two-tailed statistical probability below 0.05 was considered significant.

RESULTS

Morris water maze was conducted to evaluate memory deficit by examining average escape latency to locate escape platform in experimental rats. The rate of errors during the learning phase was high in the first trials for all groups. Nevertheless, this rate decreased significantly (P <0.001) with subsequent trials (Figure 1 A). All rats, irrespective of group, demonstrated ability to learn with an increase in rhe number of trials. We therefore conclude that the rats demonstrated the ability to learn with time, although this ability to learn differed significantly across groups (P <0.001). Intriguingly, our findings revealed that prophylactic administration of *B. coriacea*, vitamins C and E attenuated memory loss induced by $AlCl_3$. However, olive oil by itself had no significant effect on memory compared to the control rats (P > 0.05).

Additionally, we measured the level of memory retention abilities of the rats (probe test) (Figure 1B). Analysis of our results showed that memory retention was significantly disrupted or reduced by the group administered $AlCl_3$ compared to other groups either not administered $AlCl_3$ or groups treated with BC or vitamins. We found that rats in the *B. coriacea* and vitamins C and E demonstrated more crossings over the platform location than animals in the $AlCl_3$ group (P <0.001). The result suggests that *B. coriacea* was able to attenuate the probable lesions on the hippocampus, dentate gyrus and/or subiculum due to $AlCl_3$ insults, hence, the higher spatial bias among the *B. coriacea* and vitamins C and E groups.

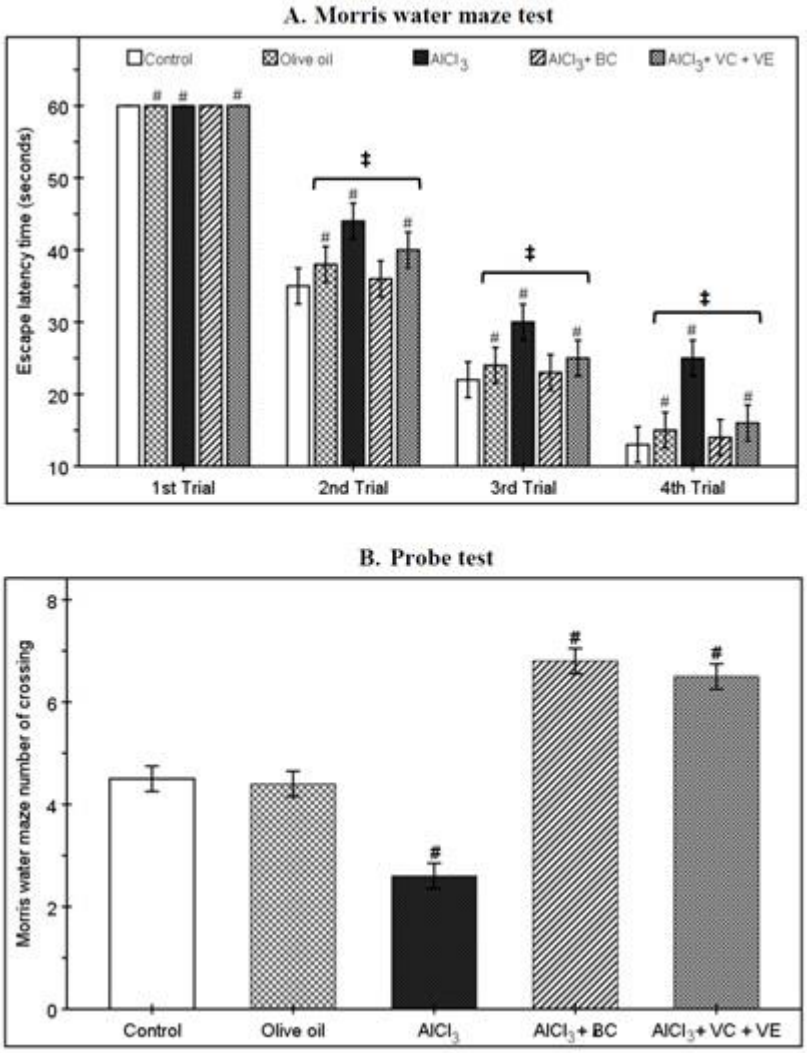

Figure 1: Effects of *B. coriacea* and vitamins C and E on $AlCl_3$-induced neurotoxicity. Rats were treated with or without $AlCl_3$ (100 mg/kg/BW/day) or co-treated with extract of *B. Coriacea* or vitamins C and E. (A): Effect of extract and vitamins on escape latency to enter the target quadrant of the Morris maze. Escape latency of treatments with different letters are significantly different at P <0.05 per trial. Escape latency significantly decreased with increase in number of trials (P <0.05). (B): Effect of the extract and vitamins on memory retention test. ‡indicates significant difference compared with 1st trial (P <0.001); #indicates significant difference compared with the control group (P <0.001).

Figure 2 represents the effect of *B. coriacea* extract on hippocampal antioxidant status in the rats. From the results, it seems that the administration of $AlCl_3$ induces oxidative damage in rats. Such as high concentrations of pro-oxidant status indicator (MDA) (Figure 2A) and a significant decrease (P <0.001) in activities of antioxidant enzymes (GSH and SOD) (Figures 2B and C). The $AlCl_3$-treated group showed a significantly higher level of MDA (P <0.001) and a significantly lower levels of GSH and SOD compared to other groups (P <0.001). Treatment with extract of *B. coriacea* and vitamins C and E resulted in a significant decrease (P <0.001) of MDA level when compared with the negative control group and a significant increase (P <0.001) in levels of SOD and GSH in the hippocampus when compare to rats in the negative control group.

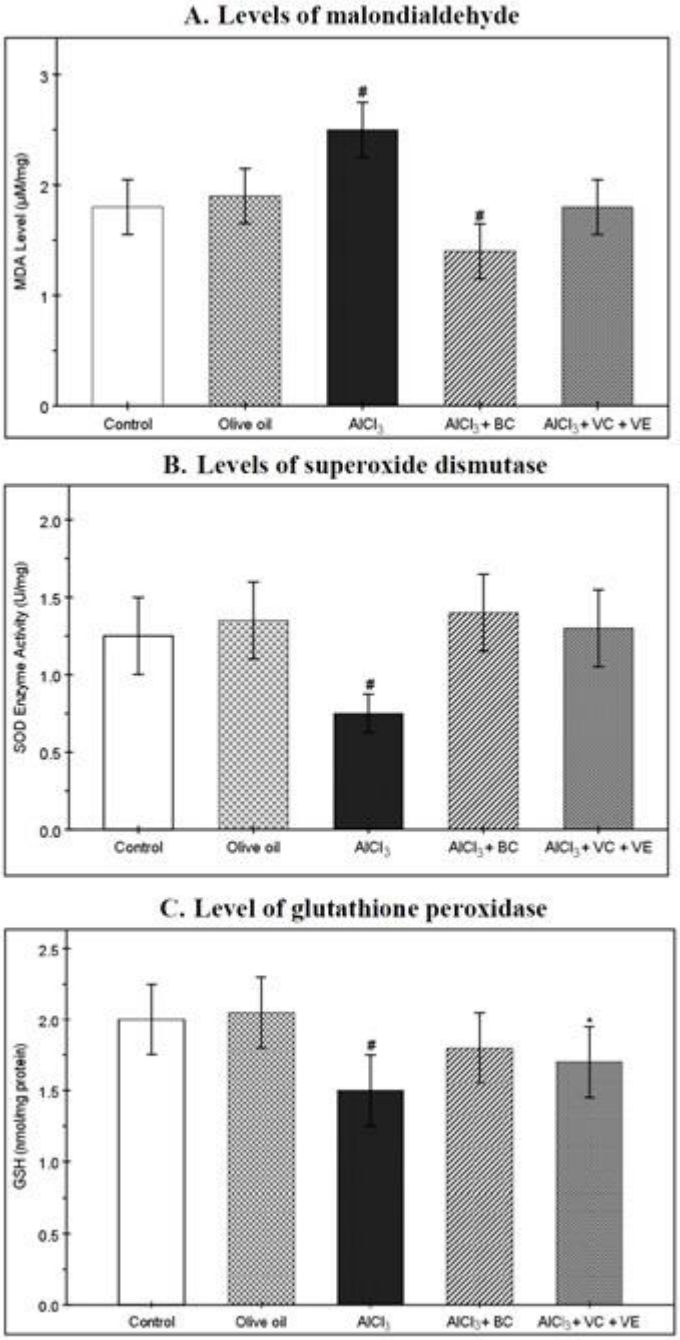

Figure 2: Effect of *Buccholzia coriacea* and vitamins C and E on oxidative stress markers in the hippocampus. In general, the administration of *B. coriacea* and vitamins C and E led to significant depletion of MDA level (P <0.001) and significant improvement in the levels of SOD and GSH levels (P <0.001). (A): MDA- malondialdehyde; (B): SOD – superoxide dismutase; (C): GSH –glutathione peroxidase.

The histological analysis of the hippocampus (at the CA1 level) of the rats in the control group, showed a normal pyramidal layer form of densely packed rounded neurons containing large vesicular nuclei. The histology of the $AlCl_3$-intoxicated group showed degenerating neurons with pyknotic nuclei, neuropil vacuolation. The plate for the olive oil group however, showed several normal pyramidal cells with few pyknotic nuclei. Also, the $AlCl_3$ + *B. coriacea* group show several normal pyramidal cells and few pyknotic nuclei. The group administered $AlCl_3$ + vitamins C and E showed several pyramidal neurons containing large vesicular nuclei, few degenerating neurons with pyknotic nuclei and neuropil vacuolation.

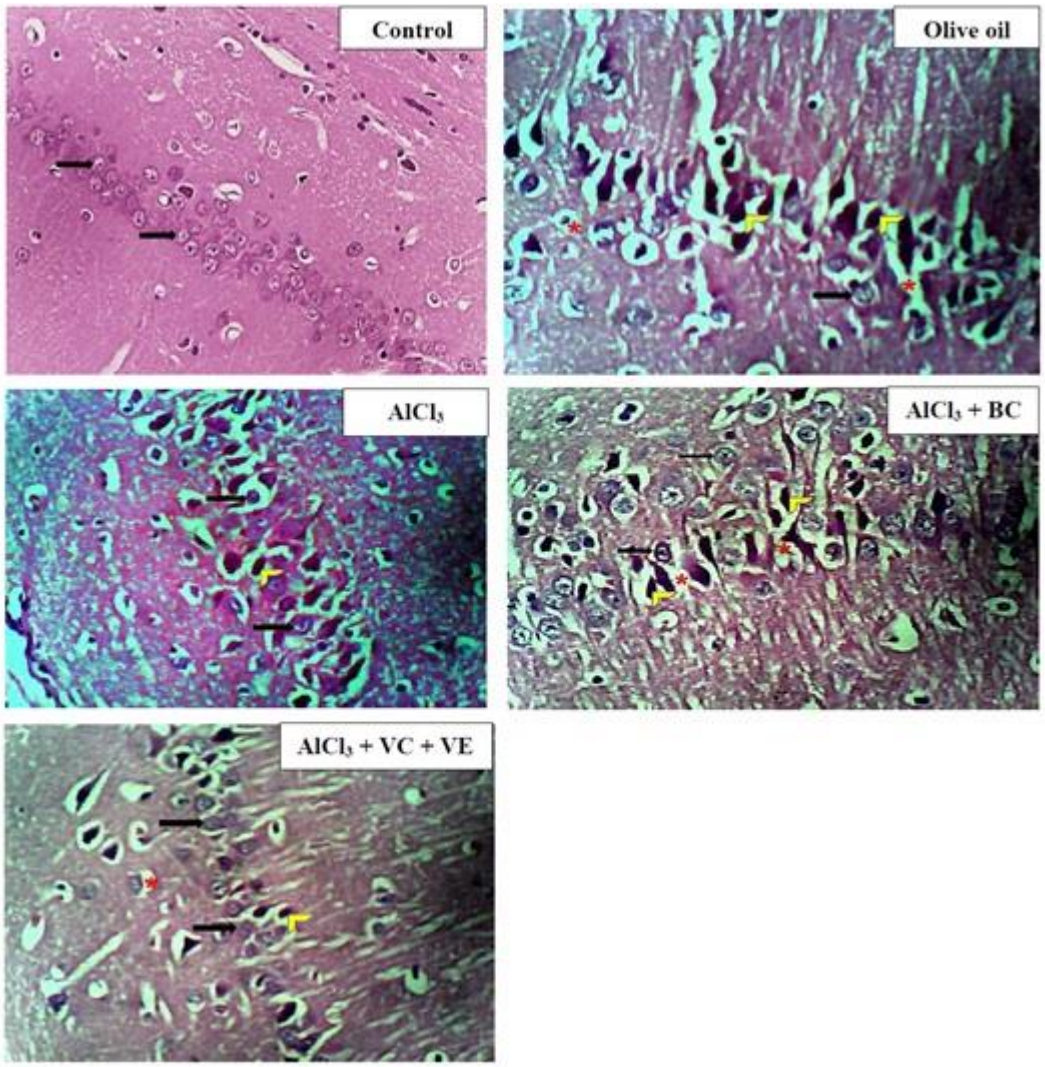

Figure 3: Photomicrographs of the histological changes in the CA1 sections of the hippocampus. H and E (× 100).

Analysis of the histology of the hippocampus (at the CA4 level) of the rats is shown in Figure 4. The control group shows densely packed rounded neurons containing large vesicular nuclei. In the $AlCl_3$ + olive oil group, there were several normal pyramidal cells. The $AlCl_3$ group showed degenerating neurons with pyknotic nuclei, neuropil vacuolation. There were several pyramidal neurons containing large vesicular nuclei, few degenerating neurons with pyknotic nuclei, neuropil vacuolation in the $AlCl_3$ + vitamins C and E groups. The $AlCl_3$ + *B. coriacea* group shows several normal pyramidal cells, very few pyknotic nuclei head and vacuolation.

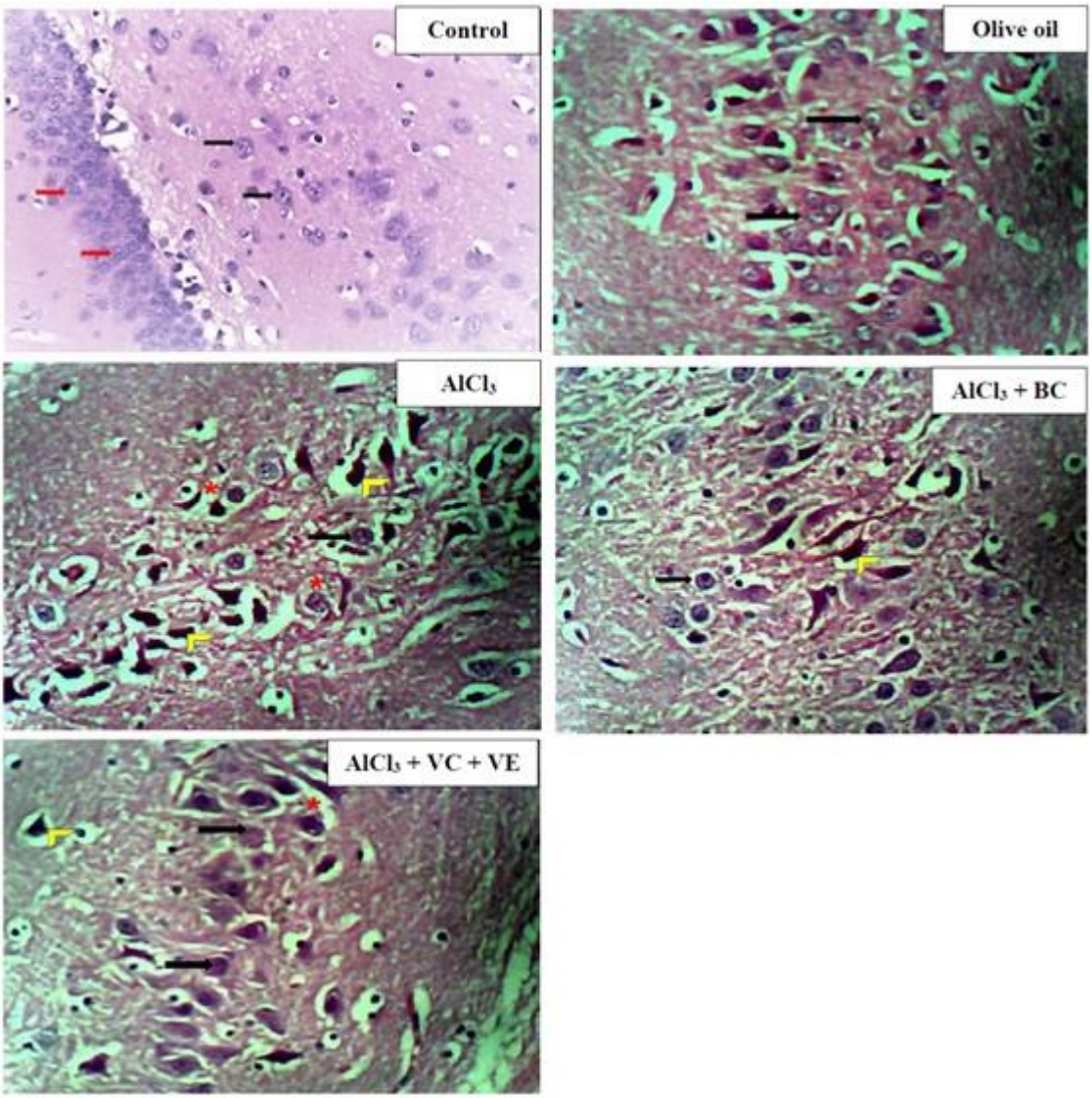

Figure 4: Photomicrographs of the CA4 sections of the hippocampus. H and E (× 100); Vitamin C+E and wonderful kola supplementation prevented brain damage and enhanced the improvement of memory performance; however, wonderful kola did slightly better.

DISCUSSION

The present study evaluated the protective an ameliorative effects of *B. coriacea* extract against $AlCl_3$-induced neurotoxicity in albino rats. Animal models are commonly used to assess neuroprotective effects of phyto-constituents that could have prophylactic effects against neurodegenerative diseases. Neurodegenerative diseases such as Alzheimer's disease are associated with abnormal cognition, and gradual pathological changes in the brain that lead to clinical symptoms of declining memory, functional capacities and subsequent physical changes. Common pathological changes of Alzheimer disease are decline and loss of neurons resulting to brain atrophy [38-40]. *B. coriacea* is popularly called wonderful kola due to its wide ethnomedicinal value for the treatment of various diseases such as high blood pressure, type 2 diabetes mellitus, influenza, infections, etc. In the present study, we investigated the ameliorative and neuroprotective effect of *B. coriacea* to prevent the deleterious effect of $AlCl_3$-induced neurotoxicity in albino rats through neurobehavioral test, measuring some oxidative stress biomarkers and histopathological evaluation of brain tissues. Administration of $AlCl_3$ induced memory disorder as evidenced in this study. However, administration of the *B. coriacea* extract and a combination of vitamins C and E prevents memory deficit by a reduction in escape latency time and increasing the number of crossings through the location of the escape platform. The decrease in escape latency and increase in the

number of crossings through the target quadrant is indicative of improved memory function [41].

B. coriacea seeds from earlier reports have been noted to enhance memory retention[42,43] by encouraging the formation of new synapses in the brain thereby improving memory and cognition. The combined supplementation of vitamins C and E have been reported to help better in retention of learning/memory as against taking them singly.

$AlCl_3$-induced neurotoxicity may be associated to neurodegeneration caused by elevated oxidative stress (Figure 2) and depletion of intracellular signal transduction pathways [44]. $AlCl_3$ inflicts its neurotoxic insults by creating an intracellular oxidative platform, a condition suitable to key complications and diseases. The results of our study showed that chronic exposure to $AlCl_3$ significantly increased the level of MDA and decreased the level of SOD and GSH. Long-term exposure to $AlCl_3$ oxidation leads to stress and changes in the antioxidant enzymes in the hippocampus. Elevated levels of MDA suggest the impact of cellular damage caused by free radicals induced by the toxicity of $AlCl_3$. Similar findings have been reported by Yuan *et al.,* who stated that AlCl3 lead to increased concentration of MDA in the hippocampus and frontal cortex of animals administered with daily dose of $AlCl_3$ via oral route[45]. Administration of *B. coriacea* elevated the levels of defensive antioxidant enzymes (SOD and GSH) with concomitant decrease in MDA, an indication of restoration of oxidative stress markers in the hippocampus. SOD plays a key role in the detoxification of the superoxide radical to hydrogen peroxide by glutathione peroxidase in lieu of GSH. The depleted level of SOD activity may have been as a result of oxidative modification of proteins. Administration of *B. coriacea* following $AlCl_3$ neurotoxicity significantly prevented the reduction in GSH levels, suggesting that *B. coriacea* is effective in preventing oxidative damage related to $AlCl_3$ exposure. Therefore, we can hypothesize that the likely surge of these enzymes could be a mechanism through which *B. coriacea* combats $AlCl_3$-induced neurotoxicity. Administration of *B. coriacea* to $AlCl_3$ treated albino rats significantly counterbalance antioxidant markers to normal levels. This promising effect of *B. coriacea* on the defense against oxidative stress is perhaps due to the phyto-constituents of its secondary metabolites, including tannins, saponins, alkaloids and flavonoids [19,20]. Previous studies have shown that because of the antioxidant properties of flavonoids, it can deplete neurotoxicity of $AlCl_3$, βA and hydrogen peroxide perhaps through antioxidant mechanism and through the inhibition of oligomerization of βA[46].

The present study showed pyknosis of hippocampal cells and necrosis of cells in adult albino rats administered with $AlCl_3$ (negative control), while the layers and cells of hippocampus of the control group showed normal histology. Researchers such as Adelodun *et al.* reported $AlCl_3$ impairment in brain architecture and function following $AlCl_3$ exposure[47]. Our study has revealed some therapeutic effect of *B. coriacea* on the hippocampus in the experimental animals induced with $AlCl_3$ toxicity. The histology of the hippocampus of albino rats in the group administered 100 mg/kg BW of AlCl3 followed by 200 mg/kg BW of ethanolic extract of *B. coriacea* for 28 days showed many viable cells with few necrotic cells. Also, the histology of the group administered 100 mg/kg/BW of $AlCl_3$ followed by 100 mg/kg of vitamin C and 1000 IU/kg BW of vitamin E also showed many viable cells with few necrotic cells. Administration of *B. coriacea* and co-administration of vitamins C and E have shown some improvement in the hippocampus when compared to rats exposed to $AlCl_3$ only. The present findings showed that *B. coriacea* and co-administration of vitamins C and E have reduced the impairment made to the hippocampus and this is in keeping with the fact that natural compounds that are rich in antioxidants help to ameliorate stress, hence alleviating the effect if oxidative compounds [48]. Adelodun *et al.* obtained similar results when they evaluated the effects of *B. coriacea* on induced neurotoxicity by $AlCl_3$ exposure in the CA3 hippocampal subfield of rats [47]. They reported no histopathological lesions sequel to treatment with *B. coriacea* for two weeks. A Study carried out by Abraham and colleagues also reported the efficacy of wonderful kola as a memory booster restoring the degenerated pyramidal and granular cells, however, scopolamine was used to induce neurodegeneration in the rats [42].

CONCLUSION

In conclusion, our findings suggest that *B. coriacea* and vitamins C and E could be beneficial in countering $AlCl_3$-induced neurotoxicity. *B. coriacea* and combination of vitamins C and E have promising prophylactic properties to be further tested as potential remedies for the treatment of diseases associated to oxidative stress, such as Alzheimer's disease.

Funding

This research did not receive any specific financial support/grant from any funding agencies in the public or private sectors.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Costa, L.G. Neurotoxicity testing: a discussion of *in vitro* alternatives, "environmental health perspectives.1998:106 (2):505 – 510.
2. Giordano, G., Costa, L.G. Developmental Neurotoxicity: Some Old and New Issues. *International scholarly Research Notices in Toxicology*. 2012: 814795.
3. Crisponi, G., Nurchi, V.M., Bertolasi, V., Remelli, M., Faa, G. Chelating agents for human diseases related to aluminium overload. *Coordination Chemistry Review*. 2012:256 (1–2):89–104.
4. Brough, D., Jouhara, H. The aluminium industry: a review on state-of-the-art technologies, environmental impacts and possibilities for waste heat recovery. *International Journal of Thermofluids*, 100007. 2020.
5. Tietz, T., Lenzner, A., Kolbaum, A.E., Zellmer, S., Riebeling C., Gürtler R., et al. Aggregated aluminium exposure: risk assessment for the general population. *Archives of Toxicology*. 2019:93(12):3503–3521.
6. Goullé, J.P., Grangeot-Keros, L. (2020). Aluminum and vaccines: current state of knowledge. *Medical Malpractice and Infection*. 2020:50 (1):16–21.
7. Krupińska, I. Aluminium drinking water treatment residuals and their toxic impact on human health. *Molecules*. 2020:25(3):641.
8. Crisponi, G, Fanni, D, Gerosa, C., Nemolato, S., Nurchi, V.M., Crespo-Alonso, M, Faa, G. The meaning of aluminium exposure on human health and aluminium-related diseases. *Biomolecular Concepts*. 2013:4 (1):77–87.
9. Exley, C. Human exposure to aluminium. *Environmental and Scientific Process Impacts*. 2013:15 (10):1807–1816.
10. Abbas, F., Eladl, M.A., El-Sherbiny, M., Abozied, N., Nabil, A., Mahmoud, S.M., et al. Celastrol and thymoquinone alleviate aluminum chloride-induced neurotoxicity: Behavioral psychomotor performance, neurotransmitter level, oxidative-inflammatory markers, and BDNF expression in rat brain. *Biomedical Pharmacotherapy*. 2022:151:113072.
11. Mehrbeheshti, N., Esmaili, Z., Ahmadi, M., Moosavi, M. A dose response effect of oral aluminum nanoparticle on novel object recognition memory, hippocampal caspase-3 and MAPKs signaling in mice. *Behavioural and Brain Research*. 2022:417:113615.
12. Hamdan, A.M.E., Alharthi, F.H.J., Alanazi, A.H., El-Emam, S.Z., Zaghlool, S.S., Metwally, K., et al. Oxidative stress induced antioxidant and neurotoxicity demonstrated in vivo zebrafish embryo or larval model and their normalization due to morin showing therapeutic implications. *Life Sciences*. 2021:283:119864.
13. Skalny, A.V., Aschner, M., Jiang, Y., Gluhcheva, Y.G., Tizabi, Y., Lobinski, R., Tinkov, A.A. Molecular mechanisms of aluminum neurotoxicity: Update on adverse effects and therapeutic strategies. *Advancements in Neurotoxicology*. 2021:5:1–34.
14. Mesole, S.B., Alfred, O.O., Yusuf, U.A., Lukubi, L., Ndhlovu, D. Apoptotic Inducement of Neuronal Cells by aluminium chloride and the Neuroprotective Effect of Eugenol in Wistar Rats. *Oxidative Medicine and Cellular Longevity*. 2020:8425643.
15. Akunne, T.C., Ilogu, L.C., Obi, B.C., Akah, P.A. Protective effects of *Buccholzia coriacea* seeds extract and fractions on blood glucose and hyperlipidemia in diabetic rats. *International Journal of Basic and Clinical Pharmacology*. 2017:6:1856.
16. Adisa, R.A., Choudhary, M.I., Olorunsogo, O.O. Hypoglycemic activity of *Buccholzia coriacea* (Capparaceae) seeds in streptozotocin-induced diabetic rats and mice. *Experimental and Toxicological Pathology*. 2011:63:619–25.
17. Nweze, N., Anene, B., Asuzu, I. Investigation of the antitrypanosomal activity of *Buccholzia coriacea* seed extract against a field strain of Trypanosoma congolense. African Journal of Traditional and Complement Alternative Medicine. 2011:8:175–80.
18. Burkill, H.M., Bouquet, A., Debray, M. Plantes medicinales de la Cote d'Ivoire. *Kew Bulletin*. 1976:31:189.
19. Obiudu, I.K., Okolie, A.C., Agbafor, K.N., Unaegbu, M.E., Engwa, G.A., Obiudu, C.V. Anti-diabetic property and phytochemical composition of aqueous and methanol extracts of *Buccholzia coriacea* seeds in alloxan-induced diabetic rats. *Journal of Medicinal Sciences*. 2015:15(5):241-245.
20. Mbata, T.I., Duru, C.M., Onwumelu, H.A. (2009). Antibacterial activity of crude seed extract of *Buccholzia coriacea* E. on some pathogenic bacteria. *Journal of Developmental Biology and Tissue Engineering*. 2009:1(1):1-5.
21. Aalikhani, M., Safdari, Y., Jahanshahi, M., Alikhani, M., Khalili, M. Comparison Between Hesperidin, Coumarin and Deferoxamine Iron Chelation and Antioxidant Activity Against Excessive Iron in the Iron Overloaded Mice. *Frontiers in Neuroscience*. 2022:15:811080.
22. Chen, S.X., Xiang, J.Y., Han, J.X., Yang-Feng, H.Z., Chen, H., Xu, M. Essential Oils from Spices Inhibit Cholinesterase Activity and Improve Behavioral Disorder in $AlCl_3$-induced Dementia. *Chemical Biodiversity*. 2022:1: e202100443.
23. Elbini-Dhouib, I., Doghri, R., Ellefi, A., Degrach, I., Srairi-Abid, N., Gati, A. Curcumin Attenuated Neurotoxicity in Sporadic Animal Model of Alzheimer's Disease. *Molecules*. 2021:26:3011.

24. Asadbegi, M., Yaghmaei, P., Salehi, I., Komaki, A., Ebrahim-Habibi, A. Investigation of thymol effect on learning and memory impairment induced by intra-hippocampal injection of amyloid beta peptide in high fat diet-fed rats. *Metabolic Brain Disease*. 2017:32:827–839.
25. Li, H., Qin, T., Li, M., Ma, S. Thymol improves high-fat diet-induced cognitive deficits in mice via ameliorating brain insulin resistance and upregulating NRF2/HO-1 pathway. *Metabolic Brain Disease*. 2017:32:385–393.
26. Javed, H., Azimullah, S., Meeran, M.N., Ansari, S.A., Ojha, S. Neuroprotective effects of thymol, a dietary monoterpene against dopaminergic neurodegeneration in rotenone-induced rat model of Parkinson's disease. *International Journal of Molecular Sciences*. 2019:20:1538.
27. Poorgholam, P., Yaghmaei, P., Hajebrahimi, Z. Thymoquinone recovers learning function in a rat model of Alzheimer's disease. *Avicenna Journal of Phytomedicine*. 2018:8:188–197.
28. Bauchi, Z.M., Kizito, D., Alhassan, A.W., Akpulu, S.P., Timbuak, J.A. (2016). Effect of Aqueous Seed Extract of *Nigella sativa* on Lead-Induced Cerebral Cortex Toxicity in Long Evans Rats. *Bayero Journal of Pure and Applied Sciences*. 2016:9(1): 48 - 52
29. Caselli, A., Cirri, P., Santi, A., Paoli, P. Morin: A Promising Natural Drug. *Current Medicinal Chemistry*. 2016:23:774–791.
30. Kuzu, M. Kandemir, F.M. Yildirim, S. Kucukler, S. Caglayan, C. Turk, E. Morin attenuates doxorubicin-induced heart and brain damage by reducing oxidative stress, inflammation and apoptosis. *Biomedical Pharmacotherapy*. 2018:106:443–453.
31. Amato, A., Terzo, S., Mulè, F. Natural Compounds as Beneficial Antioxidant Agents in Neurodegenerative Disorders: A Focus on Alzheimer's Disease. *Antioxidants*. 2019:8:608.
32. Weinstein, M., Babyn, P., Zlotkin, S. An orange a day keeps the doctor away: Scurvy in the year 2000. *Paediatrics*. 2001:108: E55-E72.
33. Li, Y., Shellhorn, H.E. New developments and novel therapeutic perspectives of vitamin C. *Journal of Nutrition*. 2007:137:2171-84.
34. Naidu, K.A. Vitamin C in human health and disease is still a mystery? *Nutrition Journal*. 2003:2:7-19.
35. Blatt, D.H., Leonard, S.W., Traber, M.G. Vitamin E kinetics and the function of tocopherol regulating proteins. *Nutrition*. 2001:17:799-805.
36. Dietrich, M., Traber, M.G., Jacques, P.F., Cross, C.E., Hu, Y., Block, G. Does alpha tocopherol play a role in the primary prevention of heart disease and cancer? A Review. *American Journal of Nutrition*. 2006:25:149-153.
37. Safieh, M., Korczyn, A.D., Michaelson, D.M. ApoE4: An emerging therapeutic target for Alzheimer's disease. *BioMed Central Medicine*. 2019:17:64.
38. Colomina, M.T., Peris-Sampedro, F. Aluminum and Alzheimer's Disease. *Neurotoxicity of Metals*. 2017:183–197.
39. McGirr, S. Venegas, C. Swaminathan, A. Alzheimer's Disease: A brief review. *Journal of Experimental Neurology* 2020:1 (3):89–98.
40. Wolters, F.J., Ikram, M.A. Epidemiology of vascular dementia. *Arteriosclerosis, Thrombosis and Vascular Biology*. 2019:39:1542–1549.
41. Wang, J., Chen, Y., Zhang, C., Xiang, Z., Ding, J., Han, X. Learning and memory deficits and Alzheimer's disease-like changes in mice after chronic exposure to microcystin-LR. *Journal of Hazardous Material*. 2019:5 (373):504–518.
42. Abraham, A., Oremosu, A.A., Shonekan, O.O., Ota, D., Okoko, I.E., Osinubi, A.A.A. Neuroprotective and Cognitive Enhancing Potential of *Buccholzia coriacea* (Wonderful Kola) Extracts Against Scopolamine-Induced Rat Model of Alzheimer's Disease-A Preliminary Study. *Journal of Experimental Research*. 2019:7(4).
43. Nwaichi, E.O., Osuoha, J.O., Monana, M.O. (2017). Nutraceutical potential of *Tetracarpidium conophorum* and *Buccholzia coriacae* in diet-induced hyperlipidemia. *Journal of Chemical Health Risks.* 2017:7:157-170.
44. Vandana, S.N., Laxman, A.K., Ninad, V.S., Nitin, R.P. Protective effect of nebivolol on aluminium-inducedneurobehavioral and biochemical alterations in rats. *International Journal of Pharmacology and Pharmaceutical Sciences.* 2014:6:7.
45. Yuan, C.Y., Lee, Y.J., Hsu, G.S.W. Aluminium overload increases oxidative stress in four functional areas of the brain of newborn rats. *Journal of Biomedicinal Science*. 2012:19:51.
46. Dibacto, R.E.K., Ambamba, B.D.A., Fils, A.E., Nyangono, C.F.B., Jules, V.K.N., Martin, F., et al. The neuroprotective effect of *Xylopia parviflora* against aluminum chloride-induced neurotoxicity in rats. *Heliyon* 2022:8:e09896
47. Adelodun, T., Ishola, O., Abijo, A., Sunday, Y., Olatunji, J., Owolabi, O., et al. Aluminium chloride-induced hippocampal damage: CA3 hippocampal subfield involvement and the neuroprotective role of *Bucholzia coriacea* ethanolic seed extract. *Phytomedicine*. 2021:1:1-11.
48. Ahamed, M., Siddiqui, M.K. Low level lead exposure and oxidative stress: current opinions. Clinica Chimica Acta Journal. 2007:383(1-2): p. 57-64.