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Ameliorative tendency of methanol leaf extract of *Anthocleista vogelli* on mercury chloride induced neurotoxicity

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Abstract
This study investigated the effects of methanol leaf extract of *A. vogelli* on some neurotoxicity indices in Wistar rats. Sixteen (16) male rats were randomly grouped into four (4); Group 1: normal control, Group 2: mercury (II) chloride, Group 3: mercury (II) chloride + Diazepam 5 mg/kg and Group 4: mercury (II) chloride + plant extract 400 mg/kg bw. Administration of extracts and drugs lasted for 21days and animals were sacrificed. Brain samples (cerebellum and cerebrum) were collected and analysed. Acetylcholinesterase, alpha-tocopherol and adenine deaminase were assayed for using standard methods. The results showed a significant ($p \leq 0.05$) increase in acetylcholinesterase activity, decrease in vitamin E concentration and adenine deaminase activity in the untreated groups relative to the control group. It was also observed that the standard drug (Diazepam) and the plant extract recorded decreased acetycholinesterase activity with increased vitamin E concentration and adenine deaminase activity significantly ($p \leq 0.05$). This goes to show that the plant extract may indeed be a potential neuroprotective agent and could thus be utilised in the management of neurotoxicity and other associated conditions.

Keywords: *Neurotoxicity, acetylcholinesterase, alpha-tocopherol, adenine deaminase, Anthocleista vogelli.*

Introduction
Plants contain many biologically active compounds which have potential for development as medicinal agents. Herbal medicines form the basis of therapeutic use in developing countries, but developed world too have joined in the use of herbal medicines.[1-2] There is a rich abundance of plants reputed in traditional medicine to possess protective and therapeutic properties.[3] It is likely that plants will continue to be a valuable source of new molecules given the wealth of complex secondary metabolites they contain. Health conditions linked to derangement in neuronal function abound. This is due largely to a surge in environmental factors that result to neurotoxicity such as mercuy.[4] This makes the search for natural products that might be able to ameliorate neurotoxicity very imperative. *Anthocleista vogelii* generally known as ‘cabbage tree’ in English because the stem of some species is unbranched or branched only at the top with huge leaves clustered at the end of the shoot[5], ‘sapo’ or ‘apaoro’ in Yoruba, ‘kwari’ in Hausa, ‘orimi’ in Benin, ‘mpoto’ in Igbo[6] and ‘odogwu’ in Igala Nigeria, are trees and shrub-like plants presently in the *Gentianaceae* family.

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A. vogelii has attracted worldwide prominence in recent years, owing to its wide range of medicinal properties. All parts of the *Anthocleista vogelii* tree leaves, flowers, seeds, fruits, roots and bark have been used traditionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders.[7]
Important phytochemicals are present in the leaf, stem–bark and root bark of *A. vogelii*.[8-9] Reducing sugar, tannin, phlobatanins, glycosides were found to be absent in both the leaf and the stem bark of the *Anthocleista* species
Other chemical compounds isolated from A.*vogelli* include: secologanin, decussatin, swertiaperennin, 1-hydroxy-3,7-dimethoxyxanthone, 7α-hydroxysitosterol, stigmasterol, hexadecanoicacid, sitosterol3-O-β-D-glucopyranoside, fagaramide and tri- terpenes.[10] *Anthocleista vogelii* Planch leaf and its constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, antihyper-glycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic, endocrine modulatory and anticarcinogenic properties.1[1-13] Given the wide range of therapeutic interventions using this plant, it is worthwhile to investigate the possible neuroprotective effect of methanol extracts from its leaves using Wistar rats. Plant products have been known to possess neuromodulation properties.[14]

Materials and methods

Preparation of plant materials

The leaves of the plant *Anthocleista vogelii* were collected from the matured plant at a farm in Umudike, Abia State. The plant sample was identified by appropriate authorities in the Department of Plant Science and Biotechnology (PSB), Michael Okpara University of Agriculture Umudike (MOUAU), Abia State, Nigeria. The plant extract was obtained following a stepwise procedure, First 500g of *A. Vogelli* leaves was collected, air dried and ground into powder. Exactly 100g of the powder was soaked in 700 mL of methanol for 72 hours, after which it was extracted and filtered into a clean beaker using a filter paper. The filtrate was then concentrated using a water bath at temperature of 40-50^{o}C until the methanol was fully evaporated leaving only the gel like green extract which was stored in the refrigerator at 4°C until used.

Experimental animals

Adult Wistar albino male rats weighing between 100–130g were procured from the animal unit of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike Abia State, Nigeria. The animals were fed with standard feed (Vital feeds finisher), and free access to water under a well-ventilated condition of 12hrs light/dark cycle. They were kept in aluminum cages and allowed to acclimatize for two weeks before the commencement of the experiment. The study was carried out in accordance with the Organization for Economic and Development (OECD) principles on Good Laboratory Practice (GLP) (OECD). Prior ethical approval (Code number, UMSE/06/012) was obtained from the ethical committee on the use of animals of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria.

Standard drugs and sample used

The standard drugs and aqueous extract of A*nthocleista vogelii* were prepared by directly weighing and dissolving the known weight of the drugs and solid extract (after evaporation of methanol) in the required volume of de-ionized water and tween 80.

Induction of neurotoxicity

Neurotoxicity was induced in each rat by administering mercury chloride (4 mg/kg body weight; orally) in distilled water. The control cohort was administered water orally. At 7days post-induction, neurotoxicity was checked for by observation method on hair loss signs and inflammation of animal body parts particularly the lower jaw.

Experimental Design

The rats were randomly divided into four groups consisting of four rats each. Group 1 (normal control) consisted of normal rats that received water and feed only. Group 2 served as the positive control (neurotoxicant control) which received 4mg/ kg body weight of mercury chloride. Rats in group 3 were induced with neurotoxicant (mercury chloride) and treated with Diazepam at a dose of 5mg/kg body weight. Animals in group 4 were neurointoxicated and treated with the plant extract at 400 mg/kg body weight. Treatments were administered orally once a day for 21 days. The untreated neurointoxicated group (Group 2) received only the vehicle (distilled water).

Biochemical analysis

AChE activity was estimated using Ellman's method.[15] Serum alpha-tocopherol was done by colorimetric method.[16-17] The Hitachi 705 discrete analyser (BoehringerMannheim, FRG) was used for the estimation of adenine deamidase; it was performed on an Acta II spectrophotometer (Beckman Instr., NY, USA) and operated according to the user protocol by the manufacturers. The surviving experimental animals were humanely sacrificed at the end of the study.

Statistical analysis

Data was presented as mean ± standard error of mean (SEM) and translated into bar charts. Statistical analysis was performed using a one way analysis of variance (ANOVA) in statistical package for social sciences (SPSS) for windows version 20.0 (SPSS Inc,Chicago II, USA).

RESULTS

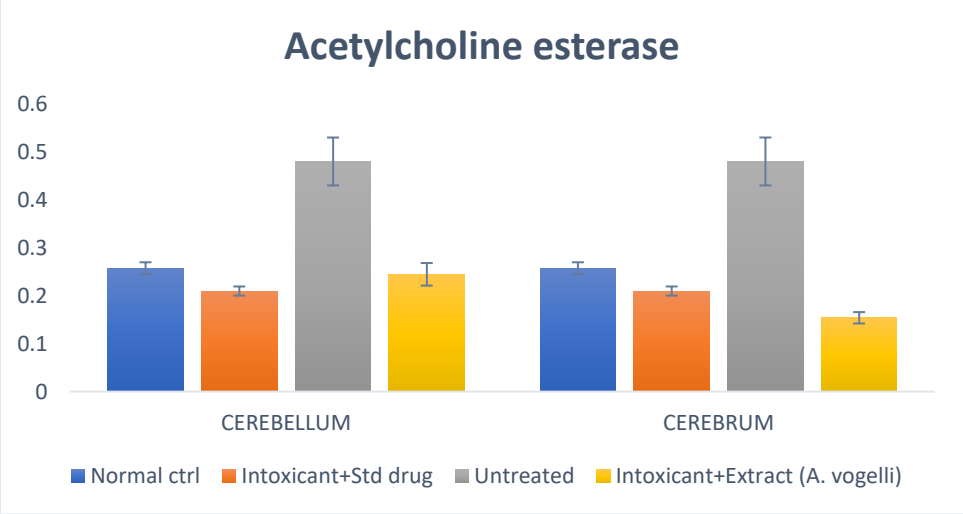

Fig 1: Effect of Methanol Extract of *A. Vogelli* on acetylcholine esterase in the cerebellum and the cerebrum of Wistar albino rat.

There was a significant ($p \leq 0.05$) increase in acetylcholinesterase activity in both the cerebrum and cerebellum of the untreated groups relative to the control group. There was also a significant decrease ($p \leq 0.05$) in AchE activity in

the group treated with the standard drug Diazepam.The extract however caused a significant decrease ($p \leq 0.05$) in AchE activity in the cerebrum but not in the cerebellum of treated rats relative to the control.

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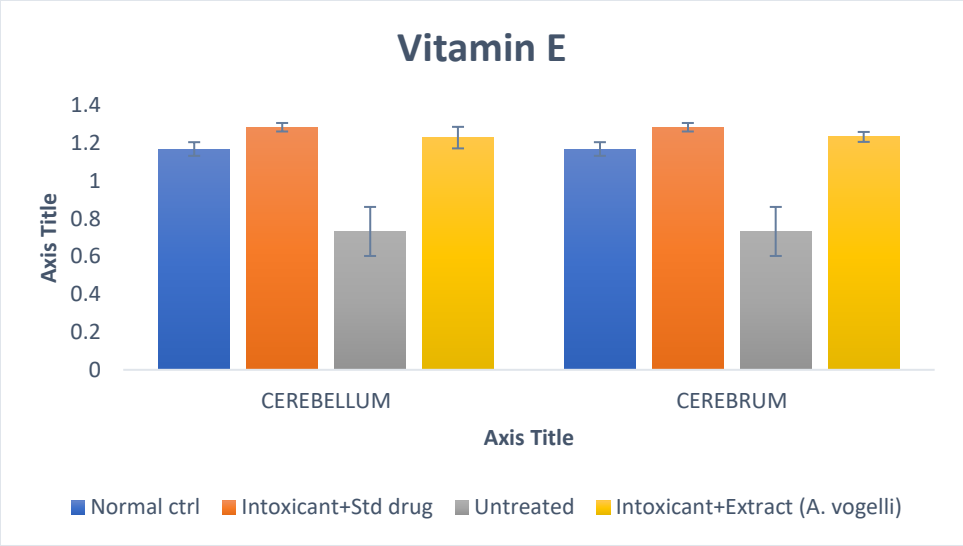

Fig 2: Effect of Methanol Extract of *A. Vogelli* on Vitamin E in the cerebellum and the Cerebrum of Wistar albino rat.

There was a significant ($p<0.05$) decrease in the concentration of vitamin E in both the cerebellum and cerebrum of the untreated group II relative to the control group I. The standard drug diazepam and the plant extract also caused a significant ($p<0.05$) increase in vitamin E concentration both in the cerebellum and the cerebrum of test animals.

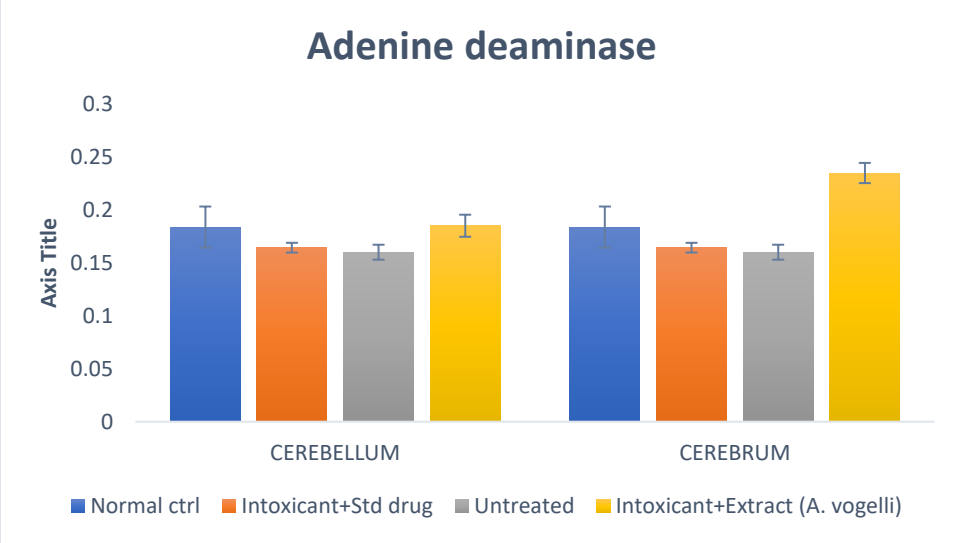

Fig 3: Effect of Methanol Extract of *A. Vogelli* on Adenine deaminase in the cerebellum and the Cerebrum of Wistar albino rat.

There was a significant ($p<0.05$) decrease in adenine deaminase activity in the untreated group and the group treated with the standard drug diazepam. The plant extract caused a significant ($p<0.05$) increase in adenine deaminase activity in the cerebrum but not in the cerebellum. While there was significant ($p<0.05$) increase in acetyl cholinesterase activity and decrease in vitamin E concentration and adenine deaminase activity in the untreated groups relative to the normal control

Discussion

The increase in acetylcholinesterase (AchE) activity observed in both the cerebrum and cerebellum of the untreated groups is indicative of the fact that mercury (II) chloride may have caused a deficiency in cholinergic neurotransmission due to a decrease in acetylcholine (Ach) levels at the synaptic cleft.[18] Because ACh is an essential player in the formation, maintenance and evocation of memory processes, excess level of Ach resulting from high AchE activity is neurotoxic and so is a low levels of Ach in the synaptic junction since it may influence memory negatively.[19]

Acetylcholinesterase is responsible for hydrolyzing the acetylcholine neurotransmitter, bringing an end point to cholinergic neurotransmission. Thus it is the primary target of a wide spectrum of compounds used as pesticides, nerve agents or therapeutic drugs for neurodegenerative diseases such as Alzheimer's disease (AD)[20], where inhibition of this enzyme attempts to compensate for the cholinergic system deficits reported not only in AD but also in other diseases such as Parkinson's disease or Myasthenia gravis[21]. Although the primary function of AChE is to terminate neural transmission, investigators have found that AChE also plays a role in neural development. Embryologically, AChE is intricately involved in the development of the nervous system and is expressed by developing neurons and during periods of axonal growth. *A. vogelli* extract and Diazepam decreased AchE activity, thereby reversing the impact of the neurotoxin; mercury (II) chloride. This shows that *A. vogelli* could be explored as a potential candidate for the treatments of Alzheimer's disease and other related neurodegenerative ailments.

Treatment of Alzheimer's disease (a prevalent disease that affects memory and cognition) has been dominated by the use of acetylcholinesterase (AChE) inhibitors. These drugs compensate for the death of cholinergic neurons and offer symptomatic relief by inhibiting acetylcholine (ACh) turnover and restoring synaptic levels of this neurotransmitter[22]. Inhibition of AChE results in a decreased breakdown and subsequent accumulation of acetylcholine. This excess acetylcholine leads to increased stimulation of muscarinic and nicotinic receptors, which provides some therapeutic relief for the memory deficits in AD[23]

Adenosine deaminase (ADA) is a ubiquitously expressed enzyme that can be found in several tissues and fluids and mediates the conversion of adenosine into inosine and of deoxyadenosine into deoxyinosine, playing a role in purine and pyrimidine metabolism.[24] ADA is considered one of the key enzymes of purine metabolism.[25] It has been observed with epithelial cell differentiation, neurotransmission, and gestation maintenance.[26] It has also been proposed that ADA, in addition to adenosine breakdown, stimulates release of excitatory amino acids and is necessary to the coupling of ADA1 adenosine receptors and heterotrimeric G proteins.[27]

The decrease in adenine deaminase activity in the untreated group and the group treated with the standard drug diazepam shows that mercury (II) choride and diazepam may have caused immunodeficiency of the brain cell through the induction of hypogammaglobulinemia. It has been reported that ADA deficiency can create several abnormalities, including vasculitis Behçet's-like disease[28] immunodeficiency due to hypogammaglobulinemia, or cytopenias.[29] Reduced activity of adenosine deaminase can also lead to pulmonary fibrosis.[30]

A.vogelli was able to reverse the toxic effect of mercury II choride by increasing adenine deaminase activity in the cerebrum but not in the cerebellum. Surprisingly the standard drug diezepam could not reverse this effect. This suggest that *A.vogelli* extract may be a potential candidate for immuno enhancement drugs.

While there was a significant ($p<0.05$) decrease in the concentration of vitamin E in both the cerebellum and cerebrum of the untreated group relative to the control group, the standard drug diazepam and the plant extract were able to reverse this effect by causing a significant ($p<0.05$) increase in vitamin E concentration both in the cerebellum and the cerebrum of test animals. Vitamin E is the collective name for a group of fat-soluble compounds with distinctive antioxidant activities.[31-32] Alpha (α-) tocopherol is the only form that is recognized to meet human requirements. The liver preferentially re-secretes only alpha-tocopherol via the hepatic alpha-tocopherol transfer protein.[33] Most of the time, vitamin E deficiency is caused by a condition where nutrients are not properly digested or absorbed; these include Crohn's disease, liver disease, cystic fibrosis, and some rare genetic disorders.[34] This means that mercury II chloride may have tampered with the digestion/absorption of vitamin E in test animals possibly through fat-malabsorption. Because the digestive tract requires fat to absorb vitamin E, people with fat-malabsorption disorders are also likely to become deficient than people without such disorders. Deficiency symptoms include peripheral neuropathy, ataxia, skeletal myopathy, retinopathy, and impairment of the immune response.[35] Vitamin E deficiency secondary to abetalipoproteinemia causes such problems as poor transmission of nerve impulses, muscle weakness, and retinal degeneration that leads to blindness.[36] Many claims have been made about vitamin E's potential to promote health and prevent and treat disease.

It was reported that vitamin E is the most important lipophilic antioxıdant and exists mainly in the cellular membranes, thus helping to maintain membrane stability.[37] Antioxidants protect cells from the damaging effects of free radicals which damage cells and might contribute to the development of cardiovascular disease and cancer.[38] Unshared electrons are highly energetic and react rapidly with oxygen to form reactive oxygen species (ROS). The body is exposed to free radicals from environmental exposures, such as cigarette smoke, air pollution and ultraviolet radiation from the sun. Vitamin E is a fat-soluble antioxidant that stops the production of ROS formed when fat undergoes oxidation. Vitamin E is involved in immune function, cell signaling, regulation of gene expression, and other metabolic processes. Alpha-tocopherol inhibits the activity of protein kinase C, an enzyme involved in cell proliferation and differentiation in smooth muscle cells, platelets, and monocytes.[32] Vitamin-E–replete endothelial cells lining the interior surface of blood vessels are better able to resist blood-cell components adhering to this surface. Vitamin E also increases the expression of two enzymes that suppress arachidonic acid metabolism, thereby increasing the release of prostacyclin from the endothelium, which, in turn, dilates blood vessels and inhibits platelet aggregation.[35] The brain has a high oxygen consumption rate and abundant polyunsaturated fatty acids in the neuronal cell membranes. Researchers hypothesize that if cumulative free-radical damage to neurons over time contributes to cognitive decline and neurodegenerative diseases, such as Alzheimer's disease, then ingestion of sufficient or supplemental antioxidants (such as vitamin E) might provide some protection.[39] Vitamin E consumption from foods or supplements was associated with less cognitive decline over 3 years in a prospective cohort study of elderly; free-living individuals aged 65–102 years.[40]

Conclusion

The ability of the plant extract to reverse the effects of mercury (ii) chloride by decreasing acetylcholinesterase activity and increasing vitamin E concentration and adenine deaminase activity is indicative of the fact that the plant extract may hold potent neuroprotective and immuno enhancement abilities which need to be harvested and exploited in the management of immuno and neurodegenerative diseases.

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Conflict of Interest
The authors declare no conflict of interest

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