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Neuroprotective Potentials of *Alstonia boonei* Extracts on Biochemical Markers of Brain Integrity in Experimental Rats

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ABSTRACT

Alstonia boonei has been proven to be a valuable source of bioactive components. Over time, these have been used in the management of various medical conditions. This work investigated the effects of *Alstonia boonei* on neuroprotective parameters/markers (vitamin E, adenine deaminase and acetylcholinesterase) in mercury chloride-induced cognitive impairment and associated oxidative damages in rats. A total of 16 adult Wistar rats (male) weighing between 100 and 130 g was divided into four groups. 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. Result shows that neurotoxicant; mercury (II) chloride (4 mg/kg body weight; orally) caused a significant decrease in vitamin E concentration and adenine deaminase activity and increased acetycholine esterase activity, the treatment with standard drug and *A. boonei* extract successfully reversed the deleterious effects of the toxicant by increasing vitamin E concentration in both the cerebrum and cerebellum, reducing acetylcholine esterase activity in the cerebrum. *A. boonei* also significantly ($p < 0.05$) increased adenine deaminase activity in the cerebrum. This indicates that the *A. boonei* extract may possess potent neuroprotective agents whose full potentials are yet to be exploited.

***Keywords*:** Cognitive, Neuroprotective parameters, *Alstonia boonei*, Acetylcholinesterase, Wistar rats.

Introduction

The brain is a complex and extremely important part of the body. It functions through neurons and neuroglia, sending out as well as receiving and interpreting simple and complex signals in the body.[1] Neurons can be injured in the course of action through various physiological mechanisms. When this happens, Acute signals are sent to the neuroglia which has the capacity and function of destroying the or repairing the injured neurons. This action represents physiological neuroprotective response by the neuroglia and helps to preserve neuronal action.[1] Neurodegeneration is a normal physiological process in brain ageing. It is equally a consequence of several neurological disorders. Cerebrovascular disease has become a leading cause of death globally with increasing prevalence.[2] Neuropsychiatric and neurodegenarative disorders abound today. These include but not limited to parkinsonism, multiple sclerosis, stroke, orofacial dyskinensis, dementia, epilepsy, cerebrovascular impairment, depression, alzheimers disease (AD) etc. These could be lifelong and life threatening.[3] Neurological disorders are usually debilitating, incapacitating, lead to loss of vital functions and reduces significantly the individual's quality of life. There is need to protect the brain and indeed the central nervous system from damage due to acute injury or as a result of physiological mechanisms.

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Strategies employed to achieve this is collectively referred to as neuroprotection.[4] Neurological disorders and injuries are associated with considerable morbidity and mortality in the short or long term. It is estimated that 52,000 fatalities arise as a result of traumatic brain injury (TBI) yearly. Again, more than 5.3 million people live with TBI-related disabilities in the USA alone.[5] The situation in the European Union is not different. About estimated 7.7 million people are said to suffer from TBI-related disabilities.[6] It is projected that the statistics from developing lacking will be grimmer when available due to a number of environmental and economic reasons.[7] There is urgent need to collate data on the impact of TBI on developing economies to enable adequate response by government and health authorities. Stroke and other TBIs are leading causes of death with increasing global burden. These are associated with high mortality and morbidity.[8-10]

Herbs have been used to manage several medical disorders since the origin of man and have continued to gain increasing utility across different human communities.[11] It is believed that herbs are repositories of natural compounds with biological activities that enable them act as anti-inflammatory and anti-oxidant agents among others.[11] The exert mechanism of action of herbs in neuroprotection has not been fully elucidated but are might be far from their ability to modulate inflammation and arrest pro-oxidant actions. There is need for further exploration of the medicinal potentials of herbs and plants as it is believed these natures healing factors have barely been scratched. Medicinal plants and herbs contain some bioactive compounds which can be used in the prevention or management of various health disorders.[12] The modern pharmaceutical industry is built on the potentials of secondary metabolites in herbs and plants.[13]

The plant *Alstonia boonei*, is a medicinal plant whose different parts are widely used to manage different health disorders in Africa.[14,15] Different names of the plant include Gods tree, *Onyame dua, Osen-nuru, or Sinduro in Twi* in Ghana.[16,17] It is also known as Australian fever bush, Australian quinine, Devil tree, Dita bark, Fever bark, or

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Palimara.[18-20] Many parts of the plant have been shown to have many beneficial properties such as antioxidant, sedative, anti-snake venom properties.[20] This present study was designed to investigate the possible neuroprotective effect of *Alstonia boonei* against mercury chloride-induced cognitive impairment and associated oxidative damages in rats.

Materials and Methods

Plant identification

The leaves of *Alstonia boonei* were collected from the matured plant at Umudike, Ikwuano local government area of Abia state in the month of June 2018. The plant sample was authenticated at the herbarium, Department of Plant Science and Biotechnology (PSB), Michael Okpara University of Agriculture Umudike (MOUAU), Abia state, Nigeria. The plant voucher number is SAPOBA 2084

Extraction of plant materials

Exactly 500 g of *A. boonei* was collected, weighed and air dried under room temperature. The dried leaves were ground and soaked in 700 mL of methanol for 48 hours, after which the filtrate collected using a filter paper. The filtrate was then concentrated with the aid of water bath at temperature of 40°C Until the methanol was fully evaporated leaving a gel-like extract. The extract was properly labelled and stored in the refrigerator at 4°C until needed.

Experimental animals

Sixteen (16) adults male Wistar rats with weighing 100 g to 130 g were used for the study. They were acquired 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 had free access to water under a well-ventilated condition of 12hrs light and dark cycle. They were kept in aluminum cages and were allowed to acclimatize for two weeks before the start 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 2001). 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.

Experimental design

The rats were randomly divided into four groups consisting of four rats each. Group 1 (normal control) consisted of rats that received feed and water only. Group 2 served as the negative control and were treated with; mercury (ii) chloride (4 mg/kg body weight; orally). Rats in group 3 (positive control) received mercury (ii) chloride (4 mg/kg body weight; orally) + Diazepam (5 mg/kg). Animals in group 4 (test group) received mercury (ii) chloride (4 mg/kg body weight; orally) + *A boonei* extract (400 mg/kg).

Induction of neurotoxicity

Neurotoxicity was induced in each rat by administering Mercury (ii) chloride (4 mg/kg body weight; orally) in distilled water. Seven days post-induction, neurotoxicity was assessed by observing hair loss signs and swollenness of animal body parts particularly the lower jaw.[21]

Assay of biochemical parameters

The activity of AChE in the brain was determined by the method described by Ellman *et al*.[22] and modified by Srikumar *et al* [23] Serum Vitamin E concentration was determined by Pearson.[24] Adenine deaminase was determined by the method of Guisti and Galanti.[25]

Statistical analysis

Data were treated with analysis of variance and mean comparison was done by Tukey post hoc test. All statistical analysis was done using Graph pad Prism for windows, version 6.01 (GraphPad Software, San Diego, California USA). P values less than 0.05 were considered statistically significant.

Results and Discussion

Methyl mercury is a known neurotoxicant responsible for many neurological alterations in humans and in animals. The mechanism of neurotoxicity is believed to be primarily due to increased generation of free radicals in the form of reactive oxygen species (ROS).[26] The significant decrease in vitamin E concentration observed in the untreated group (Figure 1), which received mercury II oxide in both the cerebellum and cerebrum shows that this substance negatively impacts vitamin E concentration in the brain and could be neurodegenerative in activity. It was also observed that *A. boonei* extract as well as the standard drug were able to restore vitamin E concentration to the normal level as obtained in the negative control group. Research has shown that adequate levels of vitamin E is critical for keeping neurons in good conditions. Vitamin E deficiency in humans leads to a pathological syndrome known as ataxia with vitamin E deficiency (AVED).[27] Individuals with this condition suffer from serious health conditions such as pigmentosis, retinitis etc.[28-30] Importantly, in such individuals, disease progression has been halted or minimized through supplementation with vitamin E.[31,32] Similar results were obtained in experimental models such as mice, rats, monkeys and horses.[29,32-35] In this study Acetylcholinesterase (AChE) activity was seen to decrease significantly in the cerebrum of animals treated with both the plant extract and the standard drug when compared to the normal control, whereas group 2, which was treated with mercury (ii) oxide alone recorded a marked increase in AChE activity as seen in Figure 2 below. However, there was no significant change in AchE activity in the cerebellum of rats administered the plant extract. It is interesting to know that drugs that reversibly inhibit acetylcholinesterase activity are today seen as having great promise for the management of Alzheimer's disease and myasthenia gravis among others. This shows that extract from *A.boonei* may be a potential candidate for the management of Alzheimer's disease.[36] The use of AChE as a biomarkers in environmental and occupational medicine has gained currency globally.[37] Again there is an upsurge in the number of environmental pollutants that inhibit aceylcholinesterase activity.[37,38] AChE has been shown to be very vital to the normal functioning of the central and peripheral nervous system.[38] Results in Figure 3 shows that there was no significant difference in Adenine deaminase activity in the cerebellum of animals in groups 3 and 4 which received the standard drug and *A.boonei* extract respectively when compared with the normal control (Group 1). However, a significant increase in adenine deaminase activity was observed in the cerebrum of animals treated with *A.boonei* extract (Group 4) relative to the normal control (Group 1).

The results showed that adenine deaminase modulated cell proliferation, survival and apoptosis of many different cell types by the control of adenosine levels.[39,40] The large degree of variation in adenine deaminase activity in the animals exposed to methylmercury suggests differences in mechanisms governing adenosine metabolism in brain and possible role together with other factors that regulates the normal functioning of the central nervous system.[41-43]

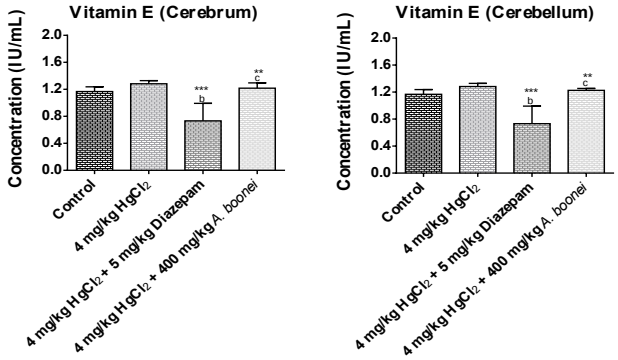

Figure 1: Effect of the treatments on vitamin E concentration in the cerebrum and cerebellum of Wistar albino rats.

a, b, and c – significant difference relative to control, 4 mg/kg $HgCl_2$, and 4 mg/kg $HgCl_2$ + 5 mg/kg Diazepam, respectively. *p < 0.05, **p < 0.01, ***p < 0.001.

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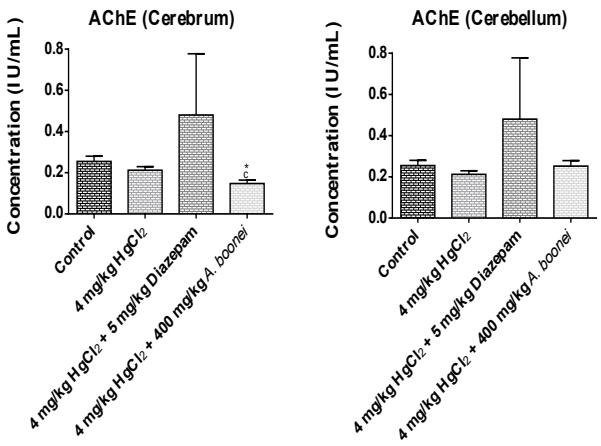

Figure 2: Effect of the treatments on Acetylcholine esterase activity in the cerebrum and cerebellum of Wistar albino rats.
a, b, and c – significant difference relative to control, 4 mg/kg $HgCl_2$, and 4 mg/kg $HgCl_2$ + 5 mg/kg Diazepam, respectively. *p < 0.05, **p < 0.01, ***p < 0.001.

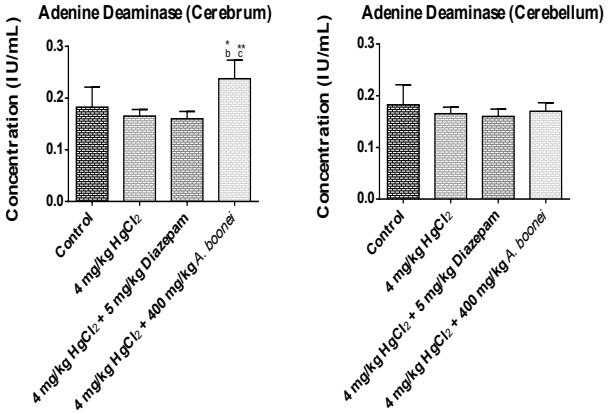

Figure 3: Effect of treatments on Adenine deaminase activity in the cerebrum and cerebellum of Wistar albino rats.
a, b, and c – significant difference relative to control, 4 mg/kg $HgCl_2$, and 4 mg/kg $HgCl_2$ + 5 mg/kg Diazepam, respectively. *p < 0.05, **p < 0.01, ***p < 0.001.

Conclusion

A. boonei leaf extract compared favourably with the standard drugs used in this study. The extract was able to restore disorder associated with depleted level of vitamin E deficiency. It reversibly inhibited the activity of acetylcholinesterase, an indication that the extract may possess bioactive components that can positively modulate neurological conditions especially in Alzheimer disease.

Conflict of Interest

The authors declare no conflict of interest

Authors Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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