Original Research Article

ALFAVACA AQUEOUS LEAF EXTRACT PROTECTIVE AND AMELIORATIVE EFFECTS ON LEAD INDUCED HIPPOCAMPUS IN WISTAR RATS

ABSTRACT

There is a claim that using medicinal plants carelessly can be dangerous. The consequences of consuming numerous of these plants over time on the brain have remained a mystery. Alfavaca is one of such numerous herbs commonly used in Nigeria. This research was done to find out how Alfavaca leaf extract affected the hippocampus of wistar rats that had been exposed to lead acetate. There were five (5) groups of twenty-five (25) Wistar rats, each with five (5) rats. Group one (1) is a control group that merely received water and a typical rat meal, the experimental group consisted of Groups 2, 3, 4, and 5. These groups received aqueous Alfavaca leaf extract at a dose of 252.98 mg/kg/day for 35 days, 180 mg/kg of lead acetate for 21 days, 126.49 mg/kg bwt of Alfavaca leaf extract for 21 days, and 180 mg/kg of lead acetate for 21 days with a dose of 252.98 mg/kg bwt for 35 days, respectively. Rats were euthanized after the treatment period. For the collected brain tissue, the hippocampus, histological, biochemical, and stereological tests were also carried out. Malonaldehyde, Superoxide Dismutase, Glutathione, and Catalase levels in the hippocampus decreased significantly when compared to control (group I), indicating that the regeneration of injured glia cells (Gliosis) was fairly evenly distributed throughout groups. The current study's findings further imply that *alfavaca* extract is a potent positive attenuator of the histo-architectural framework of a degenerating brain tissue. Similar studies on other areas of the brain are highly recommended.

Keywords*: alfavaca; Ameliorative; Cytometry; Lead acetate.*

Introduction

Brain disease in the form of brain degenerative and cerebrovascular disease has been described as one of the leading cause of human death in developing nations with about 2/1000 incidence [1]. As a result of lack of better therapeutic alternatives, dementia and stroke are mainly common with families and individual.

In most modern nations, the uses of Alfavaca are thought to be unscientific and outdated when compared to those of conventional medicines [2, 3, 4]. This is possible as a result of general belief that herbal medicine are without side effect, since they are affordable and available. A

Study conducted by Ezekwesili *et al.* [5] on phytochemical evaluation of *Ocimum gratisimum* (*Alfavaca*) shown that it contain the following properties: Alkaloid, Tannis, Flavonoids, Oligosaccharides, Eugenol etc.

The death of neurons involved a lot of events with metabolic process failure, oxidative stress and calcium loss in homeostasis [6]. During ischemic stroke, a metabolic energy decrease in the form of ATP may affect membrane ionic pumps which may cause increase in intracellular Ca^{2+} and Na^{+} concentrations and thereby increasing glutamate release [6]. The increase entry of Ca^{2+} activates enzymes such as proteases, phopholipase and oxidase that can hydrolyze the DNA molecules and destroy the cytoskeleton [7, 8]. Flavonoids compounds have been described with biological activities such as, anti-inflammatory, cytotoxic, antitumor and antioxidant activities [9, 10]. Due to the presence of apigenin, a member of the flavone class, the leaves of Alfavaca have been used for their nerve-relaxing qualities. [11]. The herb tanacetum parthenium, often known as fever few, has also been used as a preventative headache remedy. [12, 13]. In mouse cortical cultures, eugenol has previously been shown to shield neuronal cells from excitotoxicity and oxidative damage. [14]. Additionally, it protected mouse striatum from 6-hydroxydopamine (6-OHDA)-induced neurotoxicity and had a neuroprotective effect against ischemia of hippocampal CA1 neurons.-caused harm to gerbils [15]. It also demonstrated protection in rats against neuropathy brought on by acrylamide and brain toxicity brought on by chlorpyrifos, which may at least in part be related to its antioxidative stress activity [16]. Some recent findings have shown Alfavaca to be useful against gonorrheal infection, vaginitis and treatment of mental illness [17, 18]. Treatment of neurotoxicity study using Alfavaca is rare in Nigeria and Consequently, this study aims to support the usage of this plant. This study therefore aim to evaluate the histoarchitecture, cytoarchitecture, cytometrical and Biochemical changes on the hippocampus of adult wistar rats exposed to Alfavaca after injury by lead acetate with a view to check its ameliorative potential by evaluating the effects of the extracts on histology and oxidative parameters of hippocampus of an adult wistar rats.

MATERIALS AND METHODS

MATERIALS

The Materials used in this experimental study are as follow: Dissecting set, Lead acetate, Wistar rats, animal cages, fresh leaves of Alfavaca digital weighing balance, water bottles, beakers, glass slides, 1ml syringe, Orogastric tubes, Graduated Measuring cylinder set, Molten paraffin wax, Rotary microtome, Bouin's fluid, Xylene, Ehrlich's Haematoxylin, Eosin, Cresyl fast Violet stain etc.

Experimental Animals

There were 35 Wistar rats used in this experimental study which were purchased from the animal house of the Faculty of Basic Medical Sciences, Delta State University, Abraka. They were divided into five (5) groups; each group consisted of seven (7) rats. Group one (1) is control group which received standard feed and water only. Each group of animals was housed in a wooden cage of separate compartments as recommended by Oyewole *et al.* [19] and Odi *et al.*[20].

Lead acetate's source

Lead acetate of 100 percent purity, produced by BDH Chemical Ltd. in England, came from the producer the following batch number: R: 61-33-48/22-62-50.

Identification of Alfavaca leaves

Alfavaca fresh leaves were obtained from the premises of site three of Delta State University, Abraka. The Alfavaca leaves were identified and authenticated of the Department of Botany, Ekiti State University, Ado-Ekiti, Ekiti State were a specimen/herbarium number UHAE 0155 was obtained.

Ethical Consideration

The Faculty of Basic Medical Sciences at Delta State University's Ethical Committee granted approval for this study with reference number REC/FBMS/DELSU/18/33 which was conveyed through a letter as recommended by the International Society for Applied Ethology on the use and care of animals for research [21].

Preparation of Alfavaca Extract

The Alfavaca leaves obtained were taken to the Department of Pharmacology, Delta State University, Abraka for extraction. The process involved the procedure involved dried leaves

which were pulverize with a mechanical grinder. Three hundred and forty-two grams (342 g) of the powdered leaf were dissolved in 2.6 L of distilled water for 72 hours with constant stirring. The mixture was then filtered using dried Whatman's filter paper and extracted using a Soxhlet evaporator at 25 ^{0}C. The filtrate was further concentrated to dryness with the aid of a water bath set at 40 ^{0}C. The final extract, a paste-like solid (32 g) was stored in the refrigerator prior to the study.

RESULTS

Biochemical Evaluations

Effects of Alfavaca on the concentrations of MDA, GSH, CAT, and SOD in the brains of lead acetate-exposed rats

The data below illustrates how Alfavaca medication affected the levels of MDA, GSH, CAT, and SOD in the tissues of lead acetate-exposed rats' brains. Rats treated with extract of scent leaf (Group 2) had significantly lower MDA level, higher GSH, SOD and CAT level compared to the control in all the tissues examined. As opposed to the control (Group 1) and rats maintained on Alfavaca extract alone (Group 2), exposure to lead acetate alone (Group 3) significantly (P>0.05) increased MDA but decreased GSH, SOD, and CAT. The result (Group 4, 5) indicate that lead acetate exposure dramatically raised the MDA level in the brain, whereas treating lead acetate-exposed rats with high and low doses of Alfavaca helped to reduce the lead acetate-induced effects.

Table 1.0. Effects of *Alfavaca* on level of MDA, GSH, CAT and SOD in the hippocampus of lead acetate-exposed rats or lead acetate-exposed rats

Groups	MDA NmOl/L	GSH (µmol/L)	SOD (U/ml)	CAT (U/L)
1 (Control)	0.66±0.02[a]	54.5±0.97[a]	80.13±0.53[a]	3.35±0.11[a]
2 (252.98mg/kg of *Alfavaca*	0.55±0.01[a]	155.4±0.65[b]	110.24±0.67[b]	4.53±0.05[b]
3 (Lead Acetate)	3.48±0.03[b]	38.8±0. .45[c]	57.05±0.46[c]	0.15±0.01[c]
4 (Lead acetate + 126.49mg/kg of *Alfavaca*	1.68±0.03[c]	41.8±0.51[d]	86.08±0.74[d]	2.84±0.04[d]
5 (Lead acetate + 252.98mg/kg of *Alfavaca*	1.42±0.02[c]	43.4±0.93[d]	84.27±0.86[d]	3.05±0.01[d]

Values are shown as Mean SEM, with n=5. Significant differences between values in the same column with various superscripts 0.05)

Cytometry Study

The result showed a significant decrease in the pyramidal cells numbers of the prefrontal cortex among the experimental groups 3, 4 and 5 when compared with the control groups 1 and

2. The group 3 administered with lead acetate (only) showed a major reduction of pyramidal cells when compared with group 4 and 5.

There was also a significant decrease in numbers of the pyramidal cells of the among the experimental groups 3, 4 and 5 compared to those in group 1, although there was no significant difference between experimental groups 4 and 2.

Table 2.0. Cytometry Study of Hippocampus

Parameter	Group 1 (Control)	Group 2 35 days	Group 3 21 Days	Group 4 35 days	Group 5 35 Days of
Hippocampal (Pyramidal Cell)	16.65 ± 0.37^{a}	15.95 ± 0.07^{b}	8.60 ± 0.35^{c}	15.00 ± 0.17^{b}	13.36 ± 0.35^{d}

All values are expressed as Mean ± SEM. (*Alfavaca)*, L.A *(*lead acetate*).*

Photomicrograph analysis

Specifically, the cornus ammonis and the dentate gyrus are shown in their usual layers in this region of the hippocampus (Groups 1 and 2). Glial cells can be detected as well. Cell membrane seems to be unique. An illustration of the hippocampus (Group 3) showing a portion with altered cytoarchitecture and histoarchitecture. The granular and pyramidal neuron cell membranes in a portion of the hippocampus from (Group 4 & 5) were not clearly visible. Numerous oligodendrocytes with a centered nucleus and pericytoplasmic hollow were also visible.

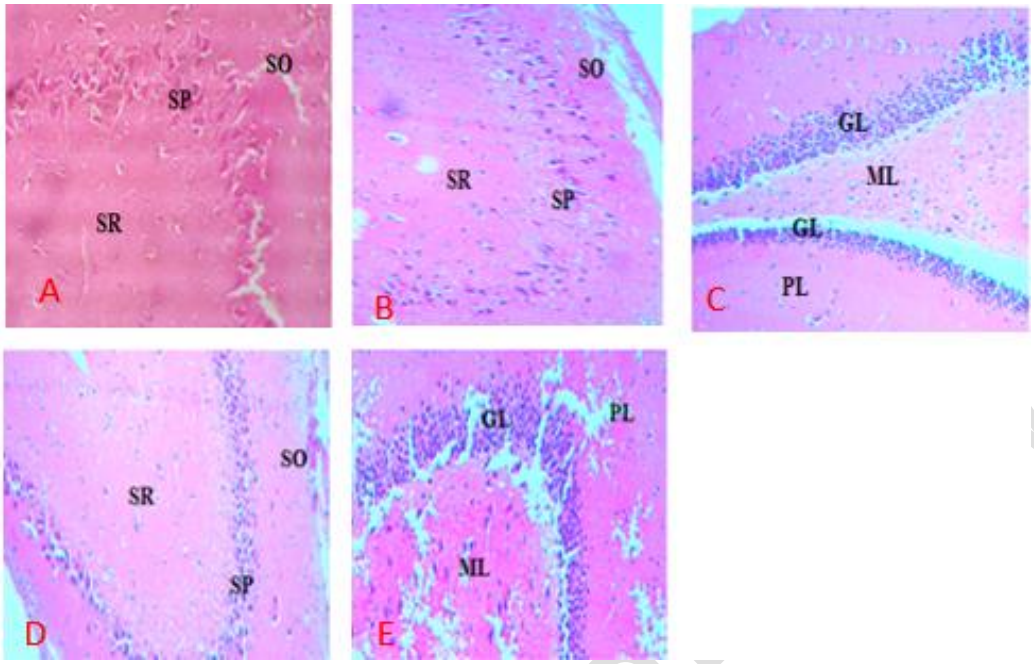

Fig. 1. Histopathological slides

A: *Section of hippocampus (control group) showing layers of the cornus ammonis and dentate gyrus.*
B *:Section of hippocampus (group 2) showing cornus ammonis with abundant pyramidal cells while the dentate gyrus with granular cells arrange in clusters.*
C: *Section of hippocampus (group 3) showing distorted histoarchitecture and cytoarchitecture.*
D: *Section of hippocampus (group 4) showing abundant pyramidal cells and many granular cells arrange in clusters.*
E*: Section of hippocampus (group 5) showing molecular, granular and polymorphic layer which were composed of neurons.*

Discussion

The current investigation revealed a significantly decreased MDA level in the hippocampus parts of the brain for all treated groups when compared with lead-acetate treated group. However, the treatment groups with Lead acetate and 'Alfavaca' were compared with control; there was a significant increase in MDA levels across groups. This is physiologically implies that treatment with Alfavaca at high and low doses, significantly improves MDA activities in the hippocampus. This finding strongly allies with those of Toscano and Guilarte [22] who found that, elevated levels of lead (Pb2+) can induce oxidative stressors, cognitive and behavioral deficits in adults and children with different brain targets by preventing the NMDAR, or N-methyl-D-aspartate receptor. Inhibition of NMDAR alters metabolic pathways involved in brain synaptic formation and neurotransmissions by significantly reducing Ca2+ entrance into the cerebral cells.

The present study also revealed GSH level caused a significantly reduction by lead acetate exposure alone, whereas treatment with high and low dosages of Alfavaca significantly increased the amount of GSH in the lead acetate-exposed rats. A similar tread was observed in the activities of SOD and CAT in the brain tissues accompanied by lead acetate induced which caused increase in membrane lipid peroxidation. The result thus confirmed the already established ability of lead acetate to induce oxidative stress in cells and tissues. Oyem *et al.* [23] had noted that lead acetate alters the cellular redox state by inhibiting the enzymes involved in antioxidant defense, such as glutathione and superoxide dismutase which function as blockers of free radical process. Most of these enzymes are metallo enzymes, which also contain sulfhydryl groups that are essential for their activities and the oxidation of these groups' results in partial or complete inhibition of antioxidant defence system, which in turn results in alteration in membrane integrity [24]. Therefore, the rise in MDA that was seen in this study after declines in SOD, CAT, and cellular GSH levels is not unexpected. It points to the toxic effects of lead acetate. These findings from this study firmly agrees with that of Ghorbe [25] who found that complex cerebral processes may be altered following dose-dependent administration of lead with minimal or no attenuation rates. The results from the present study also indicates that the extract possess strong antioxidant effects/properties which countered lead acetate induced oxidative stress. Another independent investigation revealed that taking *Ocimum gratissimum* could perhaps lessen the oxidative stress connected to degrading tissues and hence improve metabolic

activities in the disease. [26]. One of the main areas of focus of current research on diabetes mellitus is hyperglycemia, which produces reactive oxygen species (ROS) that cause oxidative stress. [26].

Data from the present study also showed a decrease in some activities of the antioxidants enzymes in rats, which are clinically useful in assaying for oxidative stress; in this case, indicative of decreased oxidative stress in any of the concerned tissue. This will also be suggestive of a decrease in the anti- oxidant defense system that ordinarily could be potent for regeneration of damaged brain tissues following duration-dependent administration of lead acetate. However, treatment with Alfavaca in any of such concerned group of rats, very minimally increased the activities of the anti- oxidant enzymes in some cases. Because oxidative stress plays a significant role in the pathophysiology of brain injury, therapies that reduce oxidative stress may be effective [27].

Histo-architectural Changes in Hippocampus

Histological results from this study were in concordance with a number of studies related to regenerative tendencies of a number of damaged tissues across different systems.

The present study administered with 180 mg/kg body weight of lead acetate shows outright distortion in histo-architectural and cyto-architectural configurations in tissues of the hippocampus. This was in agreement with the study conducted by Musa *et al.* [28], Enye *et al.* [29] and Enye *et al.* [30] on the effects of aqueous extract of Psidium guajava leaves on lead acetate induced neurotoxicity and *Mimosa pudica* on Dichlorvos hippocampal in adult Wistar rats. They observed degeneration of sub-regions (CA1, CA3 and dentate gyrus) of the hippocampus. The present study also agree with the study carried out by Ibrahim *et al.* [31]; Udi *et al.* [32], Udi *et al.*, [33] and Udi *et al.* [34] who observed neuronal damage in hippocampus and cerebellum with neurodegeneration of CA1 and CA3 regions. The present study revealed a restoring hippocampal architecture and gliosis. This was supported by a study conducted by Arhoghro *et al.* [35] and Orororo *et al.* [36]. They carried out a study on the effects of aqueous extracts of *O. gratissimum* in CCL_4 induce Liver damage in wistar albino rats. They opined that *O. gratissimum* extract inhibit and reversed carbon tetrachloride induced hepatotoxicity in rats. All this observation from the previous and present studies could explain the impaired activities of the hippocampus including storage and retrieval of information. Similarly, the hippocampus is the most medial portion of the temporal lobe cortex, where it folds first medially underneath the brain and then upward into the

lower, inside surface of the lateral ventricle. Removal of the hippocampus reportedly incapacitates victims in storing verbal and symbolic types of memories (declarative types of memory) with intermediate memory lasting beyond a few minutes. Therefore, these people are unable to establish new long-term memories of those types of information that are the basis of intelligence. This is likely to play out also with exposure to lead acetate in experimental groups if damaged hippocampus tissues are left un-regenerated, following treatment with Alfavaca extract.

Cytometry Outcome of Hippocampus Analysis

According to a cytometry study, experimental groups' hippocampal pyramidal cells significantly decreased as compared to control groups' pyramidal cells. Although there was a negligible distinction between experimental groups IV and group II. The implication of having much less pyramidal hippocampal cells, as repotted from the cytometry study, is that the hippocampus' regeneration abilities are compromised. Yet another pointer to a possible reason for low regeneration in experimental groups rats as compared with control group. This result, once more, is consistent with that of Llinas *et al*. [37] and Udi, et al. [33] who noted limited regeneration tendencies in wistar rats exposed to equivalent therapies.

Conclusion

The results of this investigation have demonstrated that alfavaca leaf extract has an antioxidant impact on hippocampal cells that are deteriorating. Damaged glia cells are also capable of significant gliosis and regeneration in a dose- and duration-dependent manner. The results of the current investigation therefore suggest alfavaca extract as a potent positive attenuator of the histo-architectural framework of a degenerating brain tissue.

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