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Neurodegenerative Potential of the Aqueous Leaf Extract of *Ocimum gratissimum*: A Histological and Biochemical Study

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ABSTRACT

Ocimum gratissimum is an herbaceous perennial shrub which is widely distributed in many regions. It is consumed in food as seasoning locally in Nigeria. In the present study, the effect of the acute administration of the aqueous leaf extract of *Ocimum gratissimum* (AeOG) on prefrontal cortical neurons was checked to assess its neurotoxicity potential. Thirty adult male Wistar rats weighing between 190-210 g were divided into 5 groups (n=6). Group A (control) received 1 ml of normal saline (p.o), groups B-E received 100, 200, 300 and 400 mg/kg AeOG (p.o) respectively. Treatment lasted for fourteen days. Twenty-four hours after treatment, animals were sacrificed and their brains were removed. The prefrontal cortices neuronal morphology was studied using haematoxylin and eosin (H&E) stain; while activities of acid phosphatase (ACP) and alkaline phosphatase (ALP) were assayed in the cerebral homogenate. AeOG administration at doses 300 and 400 mg/kg cause neuronal fragmentation and central chromatolysis with significant ($P<0.05$) increases in the activities of cerebral ACP and ALP. Our findings show that the acute use of AeOG caused neuronal fragmentation and central chromatolysis which are response to axonal injuries and may leads to onset of neurodegenerative diseases and affect cognitive and executive functions of the prefrontal cortex.

Key words: *Ocimum gratissimum*, Acid Phosphatase (ACP), Alkaline Phosphatase (ALP), Neurogenerative diseases, Rat.

INTRODUCTION

Herbs have been shown to have useful significant medicinal effects, both in their natural state, and as a source of pharmaceuticals (Memory, 2001). The use of plants in low and middle income countries has never stopped gaining popularity. In these countries, it is often the main therapeutic system majority of people resort to (WHO, 2007, Kamboj, 2000). Increasing use as oppose to enough scientific evidence on the safety of medicinal plants have raised concerns regarding the safety and detrimental effects of these remedies (Saad et al, 2006).

Ocimum gratissimum Linn (Lamiacea**)** is a shrub widely distributed in many regions. In Nigeria, it is referred to as "efinrin", "Nchonwu" and "Daidoya" respectively by the Yoruba, Igbo and Hausa tribes of Nigeria (Effraim et al., 2000). In Nigeria and most parts of West Africa, it is used as a spice and condiment in dishes, because of its high pungent flavour of clove. Traditionally, it is used locally for managing skin diseases, inflammation, insomnia, diarrhoea and liver disease (Iwu 1993, sofowora, 1995).

Phytochemical screening of the aqueous leaf extract of *O. gratissimum* (AeOG) had shown the plant to contain alkaloids, saponins, tannins, alkaloids, anthraquinone, flavonoids, steroids, terpenoids and cardiac glycosides (Holets *et al*., 2003; Akinyemi *et al*., 2005; Akinmoladun *et al*., 2007). In addition, leaves of *Ocimum gratissimum* reveal the presence

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of essential oils such as eugenol, cineole, ocimol, tetratriacontane, gratimissin, gratimissic acid and β-caryophyllene (Sainsbury and Sofowora, 1971)). The hepatoprotective (Bhakta et al, 2001), antibacterial (Perumal et al, 1998), anti-diabetic (Esposito et al, 1991), anti-tumour (Gupta et al, 2000)), laxative (Akanmu et al, 2004) and antioxidant (Luximon-Ramma, 2002) activities of essential oils in *Ocimum gratissimum* have been reported.

The present study was carried out to assess the effect the acute administration of *Ocimum gratissimum* has on integrity of prefrontal cortical neurons and cerebral phosphatase profile of adult Wistar rats. The choice of prefrontal cortex was based on its involvement in cognitive and executive functions (Glenn et al, 2009, Miller et al, 2002). It is among those brain regions having the highest baseline metabolic activity at rest and involve in decision making and behaviour (Gusnard et al, 2001).

MATERIALS AND METHODS

Preparation of extract of the aqueous leaf extract of Ocimum gratissimum

Fresh leaves of *Ocimum gratissimum* plant were purchased from a herb seller in Ilorin. Identification was done by Dr. K.S Olorunmaiye at the Herbarium of the Department of Plant Biology, University of Ilorin. The plant material was rinsed; air dried, blended and extracted using a Soxhlet extractor. The blended plant material (100 g) was placed in the Soxhlet chamber and extracted with 1250 cm^3 of distilled water. The concentrated plant material was then evaporated in an oven at a regulated temperature of 40°C.

Animal care

Thirty adult male albino Wistar rats weighing between 190-210 g were used for this study. The animals were procured from the animal house of the department of Biochemistry, University of Ilorin. The rats were housed in wooden cages under light and dark cycle at room temperature with proper aeration and cross ventilation at the animal house of the department of Anatomy, University of Ilorin, Nigeria. Prior to the experiment, the rats were allowed to acclimatize for one week. The animals were fed with pelletized feed and water was given *ad libitum*. The ethical committee of the college of health sciences, University of Ilorin reviewed and approved the procedures and experiments. Throughout the experimental period, animals were handled and maintained in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC).

Animal Grouping

The rats were divided into five groups A, B, C, D and E consisting of six animals each.

Group A (Control group): Animal in this group received 1 ml of normal saline (p.o).

Group B: Animals in this group received AeOG (100 mg/kg; p.o).

Group C: Animals in this group received AeOG (200 mg/kg; p.o).

Group D: Animals in this group received AeOG (300 mg/kg; p.o).

Group E: Animals in this group received AeOG (400 mg/kg; p.o).

Administration lasted for 14 days.

Sacrifice

Twenty-four hours after the last administration, the animals were sacrificed by cervical dislocation. Their cerebrum were excised, the prefrontal cortex dissected, fixed in 10% formol calcium solution and processed for histological examination. For enzyme studies, the whole cerebrum was quickly weighed, homogenized in 0.25 M cold sucrose solution.

Histology of the prefrontal cortex

After fixation, tissues were dehydrated and embedded in paraffin wax. 8 µm thick tissue sections were obtained using the Reichert-Jung 2050 rotary microtome; sections were stained in Haematoxylin and eosin for general neuronal outline. Images were examined under a light microscope and images captured using Olympus BH2 microscope.

Quantitative enzyme assay

After homogenization, tissue samples were centrifuged at 5000 rpm for five minutes. The

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supernatant was stored at -80°C and used for the quantitative estimation of the activities of alkaline phosphatase (ALP) and acid phosphatase (ACP) spectrophotometrically using RANDOX's kit (Antrium, UK) within 48 h.

Statistical Analysis

Data were analysed by Microsoft Excel program for windows software. Results were expressed as mean ± (S.E.M) and subjected to statistical analysis using the analysis of variance ANOVA and student's t-test. Statistical significance was set at $P<0.05$.

RESULTS

Effects of AeOG on cerebral alkaline phosphatase (ALP) levels

The oral administration of AeOG increased the cerebral levels of alkaline phosphatase across the group. This increase was significant ($P<0.05$) at doses 300 and 400 mg/kg when compared to the control group (Fig.1).

Effects of AeOG on cerebral acid phosphatase (ACP) levels

The oral administration of AeOG significantly ($P<0.05$) increased the cerebral levels of acid phosphates in a dose dependent manner when compared to the control group (Fig. 2).

Histological observation

Histological analysis of the prefrontal cortical neurons of Wistar rats exposed to AeOG reveals different changes. The histology of the control animals reveals intact neuronal arrangements with well-defined nucleus (Fig.3A). In the 100 mg/kg AeOG treated animals; the neuronal arrangements were intact and similar when compared to the control group (Fig. 3B). With increasing doses of AeOG, histological changes were seen within the neurons of the exposed animals. In the 200 mg/kg exposed groups, mild chromatolysis was noticed within prefrontal cortical neurons (Fig.3C) while in the 300 mg/kg exposed animals, neuronal swellings coupled with central chromatolysis were seen in the neurons (Fig.3D). In the 400 mg/kg exposed animals, dying neurons with distorted cytoarchitecture and central chromatolysis were seen (Fig.3E).

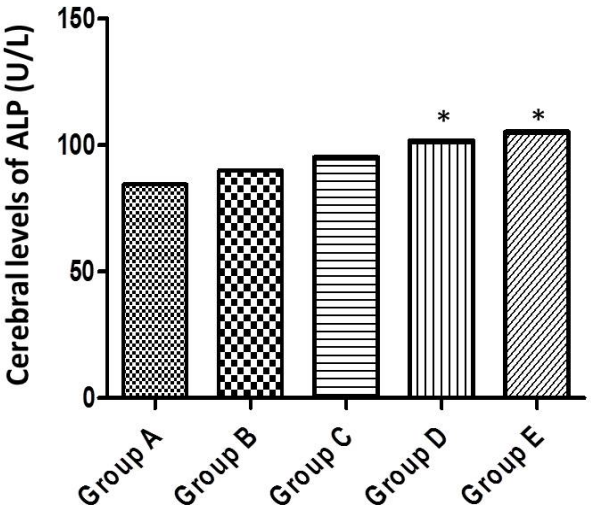

Figure 1: Shows effect of AeOG on the activity of cerebral alkaline phosphatase (ALP)

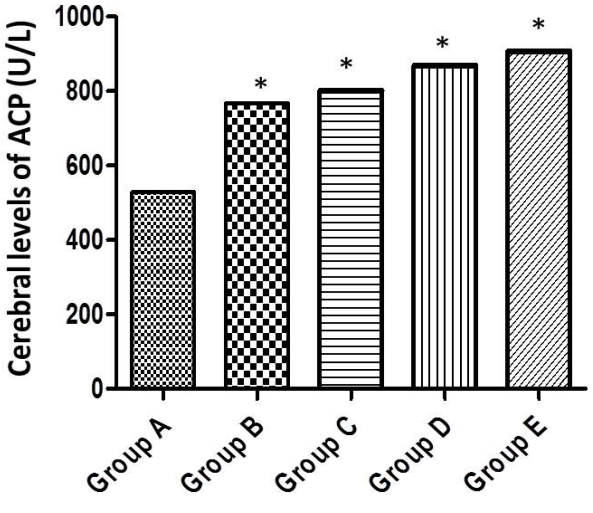

Figure 2: Shows effect of AeOG on the activity of cerebral acid phosphatase (ACP)

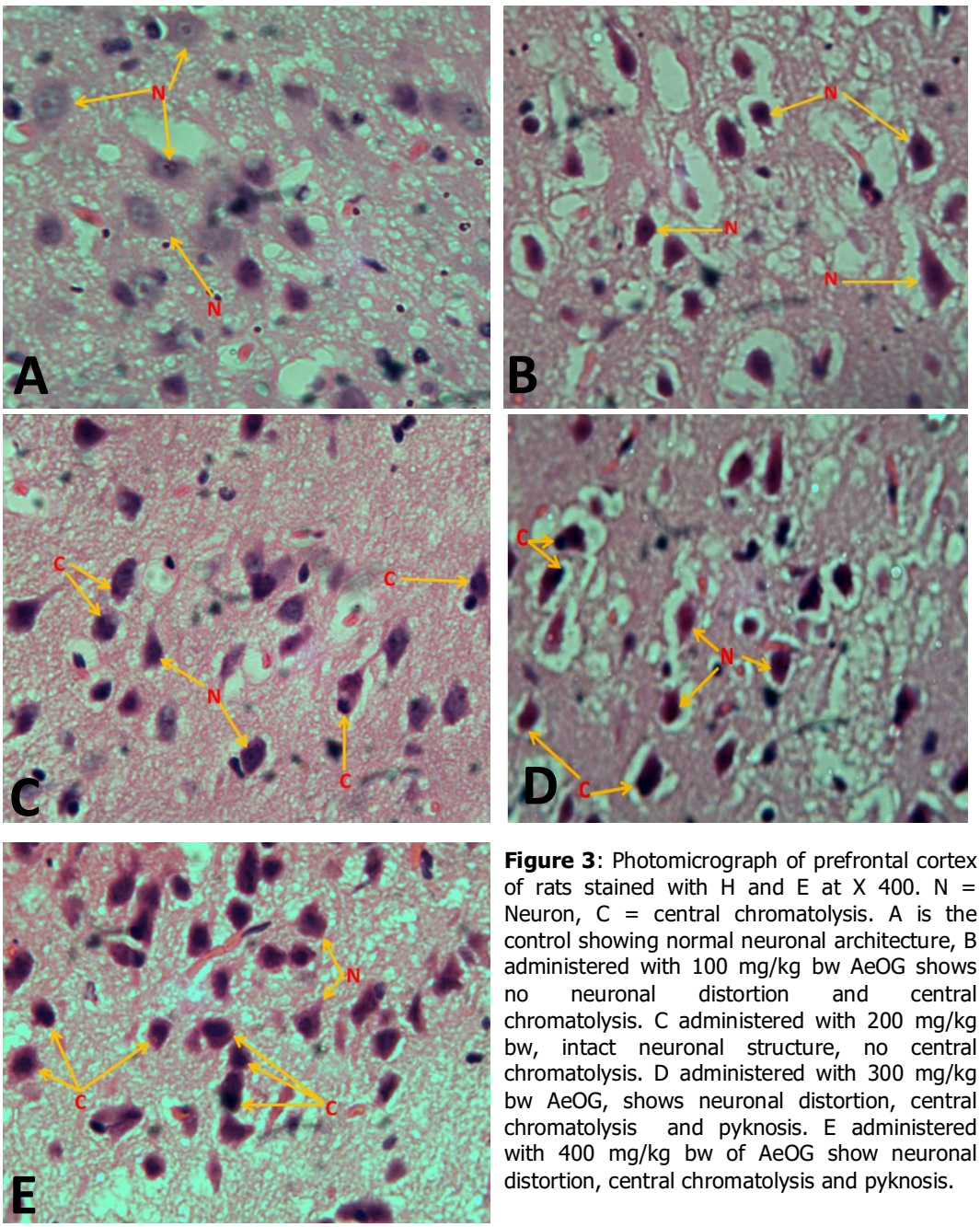

Figure 3: Photomicrograph of prefrontal cortex of rats stained with H and E at X 400. N = Neuron, C = central chromatolysis. A is the control showing normal neuronal architecture, B administered with 100 mg/kg bw AeOG shows no neuronal distortion and central chromatolysis. C administered with 200 mg/kg bw, intact neuronal structure, no central chromatolysis. D administered with 300 mg/kg bw AeOG, shows neuronal distortion, central chromatolysis and pyknosis. E administered with 400 mg/kg bw of AeOG show neuronal distortion, central chromatolysis and pyknosis.

DISCUSSION

Acid phosphatase is an enzyme of the lysosomal membrane (Zhang et al., 2009).
ACP was chosen for assay on the basis of its specificity for lysosomal membrane. It gives a picture of the sequence of cell damage if any after exposure to insults resulting from chemical compounds (Chu and Lin, 1998).

The increase in ACP activity observed following administration of AeOG could be a consequence of *de novo* synthesis induction by accumulation of saponins, one of the phytochemicals in the extract, in the brain. Saponins have been implicated to have complexation with cholesterol to form pores in cell membrane bilayers like in red cell membranes, where complexation leads to haemolysis on intravenous injection (Francis et al, 2002). Another possible explanation is the release of the enzymes from the brain lysosomal membrane, which is an indication of tissue damage.

The increase in the activities phosphatases (ALP and ACP) in the groups treated with AeOG as shown above is an indication of neurotoxic effect of the extract. These results were consistent with Obianime et al, (2011) who reported a significant increase in total acid phosphatase and prostatic acid phosphatase after administration of crude extract of ocimum gratissimum. For ACP, this may occur as a result of increase in the synthesis of the enzyme by Golgi-lysosomal system of the phagocytes into the digestive vacuoles in response to cell death or pores in the membrane that may be triggered by stress or hyperstimulatory activity of the extract (Al-Ali and Robinson, 1982).

Histological observations revealed normal neuronal outline with intact cellular architecture in control animals (fig 3). The central chromatolysis observed in the animals exposed to 300 and 400 mg/kg AeOG conformed to an increase activity of ACP (Fig 3D and E). This is characterised by widely spaced, distorted, enlarged neuronal cells and central chromatolysis which are indications of axonal injuries. This indicates the neurodegenerative potential of AEOG at high doses.

Recent studies have proven that AeOG has hepatoprotective, antioxidant (Rabelo *et al*.,2003; Odukoya *et al*., 2005; Leal *et al*., 2006; Aprioku and Obianime, 2008) and antibacterial effects due to presence of antioxidants such as flavonoids and polyphenols in the extract (Effraim el al, 2000, Abdulazeez et al, 2013)). It has also been reported to supresses the hematopoietic system an attribute linked to its presence of saponin (Jimoh et al, 2009). From these reports, it may be concluded that AeOG has both antioxidant and oxidant properties, depending on the tissue/organ system under investigation or the duration of administration. From the phytochemical analysis of AeOG, it can be easily deduced that it has complex and large amounts of secondary metabolites which include oxidants (saponins, triterpenes and alkaloids) and antioxidants (eugenol, flavonoids, citral, linalool).

Results from the present study reveal that AeOG has neurodegenerative potential especially at high concentration. AeOG cause no significant neurodegenerative changes in Wistar rats at low doses (Fig 3B and C), while in high doses it was toxic to prefrontal cortical neurons (Fig 6 and 7). The reason was probably that AeOG has multiple actions, such as antioxidation (Nwanjo and Oze, 2006, Akinmoladunn et al, 2007) thus inducing apoptosis.

In conclusion results from our study reveal that the aqueous leaves extract of ocimum gratissimum has neurodegenerative potential initiated by membrane malformation and lysosomal activity. The neurodegeneration was found to be dose dependent. Though, ocimum gratissimum is a popular condiment/seasoning and herbal medicine in Africa, its chronic usage is not advisable for sound brain activity.

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