Morpho-Functional Changes in Cerebellum of Lead II Acetate Exposed, *Ocimum gratissimum* Administered Wistar Rats

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Authors’ contributions

This work was carried out in collaboration between all authors. Authors AAA and MAO designed the study. Author KEA wrote and monitored the manuscript. Author SEI supervised the entire work. All authors read and approved the final manuscript.

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ABSTRACT

In the human nervous system, available studies assert; that the Neuroglia cells (special neurons in the brain) may provide structural support and/or regenerative tendencies (gliosis) to damages within the circuitry. This study conducted to investigate the activity of *Ocimum gratissimum* leaf extract on the regenerative functions of the cerebellum of lead II acetate exposed wistar rats. A total of twenty five (25) Wistar rats were divided into five (5) groups of five (5) rats each. With Group one (1) receiving standard rat diet and water only (control group), Groups 2, 3, 4 and 5 respectively got 252.98 mg/kg/day of aqueous *O. gratissimum* leaves extract for 35 days, 180 mg/kg of lead II acetate for 21 days, 180 mg/kg of lead II acetate for 21 days with continued treatment of 126.49 mg/kg of *O. gratissimum* leaf extract for 35 days, and 180 mg/kg of lead II acetate with continued treatment of 252.98 mg/kg of *O. gratissimum* leaf extract for 21 days. Rats were then euthanized.
(after treatment period), with blood samples obtained and assayed for biochemical changes. Histological and stereological examinations were also conducted for cerebella tissue. Upon comparison with control (group I), one-way analysis of variance (ANOVA) returned a significantly high GSH value for group one rats as compared to other groups. Even though huge degeneration was seen for group III rats, Cerebella Malonaldehyde, Superoxide Dismutase, Glutathione and Catalase levels, were relatively low across groups IV and V as against the control. Histological section through group I rats showed cerebella purkinje layer appearing deeply basophilic, with a characteristically condensed Nissl granule, thus describing a normal architecture of the cerebellar cortex. Pyramidal and granular axonic and dendritic cells were also seen to have embedded neuropils within group II rats. Conclusively, Study has shown that O. gratissimum extract consumption may be potent for low to minimal anti-oxidant effect, and regenerative tendencies (gliosis) on the cerebellum. More sophisticated but similar studies are recommended for other areas of the brain.

**Keywords:** Ocimum gratissimum; prefrontal cortex; oxidative stressors.

1. **INTRODUCTION**

Lead taken internally in any of its forms is highly toxic; with symptomatic effects after prolonged accumulated in the body. Such symptoms of lead poisoning may be seen as anemia, weakness, constipation, colic, palsy, and often a paralysis of the wrists and ankles. Lead has been investigated to reduce intelligence, delay motor development in the brain, impair memory, and cause hearing problems and troubles in balance. Present-day treatment of lead poisoning includes the administration of calcium disodium Ethylene Diamine Tetra Acidic acid (EDTA), a chelating agent that displaces calcium from blood to form a stable complex [1-3].

As an alternative of orthodox therapy to lead poisoning, medicinal plants have often been implicated by inhabitants of developing countries. In Africa for instance, hundreds of traditional medical practitioners (Trado-Medics) explore roots and herbs in the management and/or control of different ailments; even though only few of such plants have received scientific investigation.

*Ocimum gratissimum* (scent leaf) is one of such of relegated herbs by the “creams” of research community. Its extract is widespread across tropical nations like Brazil, where it is widely known as “alfavacão, alfavaca and alfavaca-cravo” [4]. It is often used in traditional medicines across South America and Africa for the management of a variety of ailments. Such conditions as bacterial infections, diarrhoea, diabetes [5], respiratory-tract infections, pneumonia, fever and coughs [6]. Studies have evaluated some biologic activities of *O. gratissimum* extracts to include anti-diarrheal effects [7], high antiviral indices against HIV-1 and HIV-2 [8], improved immunobiological activity to phagocytic functions without affecting the humoral or cell-mediated immune system [9], as antifungal agent [10], and as a relaxant on isolated ileum from guinea pig [10]. In fact, one of its essential oil component, eugenol, has been shown to be a potent inhibitor of *Haemonchus contortus*, a gastrointestinal parasite of small ruminants [11].

*O. gratissimum* flowers and leaves are rich in essential oils, and thus, used in preparation of teas and infusion [2]. In the coastal areas of Nigeria, the plant is used in the treatment of epilepsy, high fever and diarrhoea [3]. In the Savannah areas, decoctions of the leaves are used to treat mental illness [12]. *O. gratissimum* is used by the Ibo’s of South eastern Nigeria in the management of baby’s cord, to keep the wound surfaces sterile. It is also used in the treatment of fungal infections, fever, cold and catarrh [13].

Currently, an estimated 15% of the world’s population consumes *O. gratissimum*-containing substance daily [13-14]. Given this widespread use, its potential health effects are important for public health as well as for helping individuals make informed choices regarding its consumption.

As controversies on its consumption rages, several reports have found it to produce negative (undesirable) and/or positive (desirable) effects. Its health effects have been, and are still being extensively investigated, especially in Africa [14]. *O. gratissimum* is known to pose some beneficial antioxidant effects as a result of its component ingredients; Alkaloids, Tannin, Saponin and
Flavonoids [15-16]. Present study was therefore designed to determine the effect of O. gratissimum on the cerebellum of lead II acetate exposed adult male wistar rats. The study investigated its effect on oxidative stress markers (Catalase, Malonaldehyde, Glutathione and Superoxide Dismutase) as a prelude to understanding its possible role in regeneration of degenerated brain cells (cerebellum), following its duration and dose-dependent administration to lead II acetate exposed rats.

1.1 Aim of Study

Study investigated the activity of O. gratissimum leaf extract on the regenerative functions of the cerebellum of lead II acetate exposed wistar rats. Study also evaluated the effect O. gratissimum in the amelioration of free-radicals that could result from cerebellar damages due to exposure to lead II acetate in adult Wistar rats. Again, study investigated the histo-architecture changes in cerebellum due to O. gratissimum extract administration.

2. METHODOLOGY

2.1 Scope of Study

Study was conducted in the Department of Anatomy and Cell Biology, Faculty of Basic Medical Sciences, Delta State University, Abraka, Delta State, Nigeria. Due to the sensitivity and invasive nature of the study, Wistar rats were chosen as experimental model.

2.2 Study Design

Twenty five (25) wistar rats were purchased from the animal house of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Delta State. They were then housed and acclimatized in wooden cages for a period two (II) weeks; after the acclimatization period, animals were divided into five (5) groups of five (5) rats each. While Group one (1) received standard rat diet and water only (control group), Groups 2, 3, 4 and 5 respectively got 252.98 mg/kg/day of aqueous O. gratissimum leaves extract for 35 days, 180 mg/kg of lead II acetate for 21 days, 180 mg/kg of lead II acetate for 21days with continued treatment of 126.49 mg/kg of O. gratissimum leaf extract for 35 days, and 180 mg/kg of lead II acetate with continued treatment of 252.98 mg/kg of O. gratissimum leaf extract for 21 days.

2.3 Resources and Sources

2.3.1 Collection and identification O. gratissimum sample

Fresh O. gratissimum (scent leaves) were obtained within the premises of site three of the Delta State University, Abraka. They were then identified and authenticated by expert taxonomists from the Department of Botany, Delta State University, Abraka.

2.4 Preparation of Plant's Extract

Obtained O. gratissimum (scent) leaves was extracted in the laboratory of the department of pharmacology, Delta State University, Abraka. At a temperature of 30 ± 2°C, the fresh scent leaves were air-dried and pulverized with mechanical grinder; with obtained powders stored until needed. Next, 50 g of the powder was extracted with distilled water of 500 mls (via maceration) for 48 hrs, with mixture decanted and filtered with sterile whatman paper. Preparation of stock solution (500 mg/ml) was then made from weighed samples (20 g in 10 ml distilled water) of the extract as previously described by Eno et al. [8]. The stock solution was then labelled and refrigerated at 4°C for future use.

2.5 Acute Toxicity (LD50) Determination

As recommended by Mohammed et al. [12], the LD50 for oral administration of aqueous O. gratissimum leave extract on Wistar rats is 1264.9 mg/kg, with safe dose being 1/10 of LD50 as recommended by Neharkar and Gaikward [13]. Therefore, doses of 10% (126.49 mg/kg bwt) and 20% (252.98 mg/kg bwt) of LD50 were adopted and used in this study. For lead II acetate, LD50 for its oral administration to wistar rats as recommended by Neharkar and Gaikward [13] is 600 mg/kg, with safe dose being 1/10 of LD50. This study used a 30% (180 mg) dose for lead II acetate on experimental rats.

2.6 Ethical Considerations

Ethical clearance was obtained from the Bioethics and Research Committee of the Faculty of Basic Medical Sciences, Delta State University. This was conveyed through a letter as recommended by the International Society for Applied Ethology on the use and care of animals for research.
2.7 Mode of Administration

Both lead II acetate and O. gratissimum leaves extract were orally given to rats with the aid of a 1ml syringe, having an in-tube sterile cannula. The time of administration was between 8:00 am and 10:00 am daily as recommended by Ejebe et al. [14].

2.8 Procedure

For each group, animals were weighed with digital balance before administration of test substances. Weighing occurred daily (and on the day of sacrifice) during extract administration.

2.9 Sample Collection

Rats were euthanized at the end of the entire process by cervical dislocation. A 5 ml syringe and 23 G needle was then used to obtain blood samples via cardiac puncture. By way of centrifugation, serum was then obtained (at 3500 rpm) in 10 minutes for biochemical analysis. Cerebella tissues were also harvested and stored in a 10% formol-saline container and subjected through fixation for histological photomicrography.

2.10 Histological Procedure

2.10.1 Manual processing of tissues

Tissues from harvested prefrontal cortex were grossed and placed in pre-labelled tissue it was thereafter fixed in bouin’s fluid and dehydrated in increasing grades of alcohol concentration (70%, 90%, and 100%) for 2 hours and then cleared in xylene for another 2 hours. After clearing, tissues containing cassette was infiltrated in paraffin wax for 2 hours each and stirred every 10 minutes with the wax placed in an oven at a regulated temperature of 55°C to keep it in molten state.

Casting and embedding then followed with the help of metallic moulds. Metallic base moulds were placed on a metallic plate, which acted as a base of the mould and molten wax was poured into it. Next, the tissues were placed in the mould filled with wax and left to solidify and cool in a refrigerator. After solidification the blocks of the wax were removed and labelled for microtomy.

2.11 Sectioning and Mounting of Cerebellar Tissues

Cerebellar tissues were sectioned with a rotary microtome at 5 microns (µ). For purpose of removing available creases and wrinkles, obtained sections were flattened in a water bath, with slides used to pick out ribbons. They were then drained and heated on histologically hot plate at 60°C. Next, two variants were then made out of the sections and respectively stained with Cresyl Violet (a more specific dye) and haematoxylin and eosin (H&E). Each were then, mounted on Distrene-(a polystyrene), a plasticizer (tricresyl phosphate) and Xylene (DPX) treated slides. With the DPX acting as adhesive for the sections on the slides, the sections were then allowed to dry and examined first under the low power and then high power magnification of the light microscope.

2.12 Biochemical Analysis

Following period of experimentation, animals were euthanized via cervical dislocation. Cerebellum was harvested, washed, crushed and homogenized in a solution of KCl. Homogenate was then diluted and centrifuged, with supernatant subjected to decantation and analysed for the following antioxidant enzymes:

2.12.1 Super Oxide Dismutase (SOD)

SOD enzyme activity was determined according to the method of Kono [15]. The method measured its ability to inhibit auto-oxidation of epinephrine, and accessed through the activity of acetyl cholinesterase enzyme as recommended. Enzyme activities were expressed as SOD units, where one unit of SOD is defined as the quantity of enzyme needed to inhibit fifty percent (50%) epinephrine per minute, per milligram of protein at 25°C and pH 7.8.

2.12.2 Catalase

Catalase activity was determined in accordance with the method of Luck [16] who measured the initial rate of hydrogen peroxide's (50 mM) decomposition at 240 nm. Here. Results were expressed in units/mg of protein; with one unit being the amount of enzyme that hydrolyses 1 µmol of hydrogen peroxide per minute per milligram of protein at 30°C.

2.12.3 Glutathione (GSH)

By method of Moron et al. [17], Glutathione activity was determined using 5, 5-dithio-bis-2-nitrobenzoic acid (DTNB). Here, 100 µl of sample was added to 1 ml of 0.2M Tris-EDTA buffer, pH 8.2. 0.9 ml of 20 mM EDTA, pH 4.7 was added 20 ul of 10 mM DTNB was added and the sample was allowed to incubate at room temperature. The mixture was centrifuged and the absorbance of the supernatant was read against distilled water.
water at 412 nm. Calculation was made using: GSH = OD \times \frac{V}{v}, where OD = absorbance; = extinction coefficient; V = total volume of reaction mixture; and v = volume of sample in reaction mixture.

### 2.1.2.4 Malondialdehyde (MDA)

The activity of Malondialdehyde was determined by assaying for the quantity of the Thio-Barbituric Acid (TBARs).

### 2.1.3 Statistical Analysis

Obtained data were expressed as mean ± standard deviation. Stained slides were interpreted with the aid of a light microscope and observed for both histological and morphological changes across the groups. Statistical significance for obtained variables was calculated with the one-way analysis of variance (ANOVA). Differences between means were considered at p < 0.05.

### 3. RESULTS

Tables and figures below represents obtained results after careful observation and analysis.

### 4. DISCUSSION

In traditional medical practice, *O. gratissimum* has been implicated in treatment of malaria, convulsion, bacterial and fungal infections, in epilepsy, as well as in mental illnesses [17-18]. In some part of Niger Delta (Nigeria), it is applicable as anti-diarrhoea. Though little is known about its effects on the brain [19]; the activity of its leaf extract on the cerebellum is examined in current study.

#### Table 1. Cerebellar changes in GSH level for *O. gratissimum* fed, Lead II acetate exposed rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Control)</td>
<td>47.3±2.01\textsuperscript{a}</td>
</tr>
<tr>
<td>2 (Scent Leaf)</td>
<td>88.2±3.89\textsuperscript{b}</td>
</tr>
<tr>
<td>3 (Lead Acetate)</td>
<td>29.8±1.98\textsuperscript{c}</td>
</tr>
<tr>
<td>4 (Lead acetate + low dose Scent leaf)</td>
<td>36.5±4.05\textsuperscript{d}</td>
</tr>
<tr>
<td>5 (Lead acetate + high dose Scent leaf)</td>
<td>36.8±3.59\textsuperscript{d}</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD. n=4. Values on the same column with different superscripts differ significantly (p < 0.05)

#### Table 2. Cerebellar changes in MDA level for *O. gratissimum* fed, Lead II acetate exposed rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (nmol/gm tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Control)</td>
<td>1.02±0.09\textsuperscript{a}</td>
</tr>
<tr>
<td>2 (Scent Leaf)</td>
<td>0.44±0.03\textsuperscript{b}</td>
</tr>
<tr>
<td>3 (Lead Acetate)</td>
<td>3.36±0.10\textsuperscript{c}</td>
</tr>
<tr>
<td>4 (Lead acetate + low dose Scent leaf)</td>
<td>1.74±0.04\textsuperscript{d}</td>
</tr>
<tr>
<td>5 (Lead acetate + high dose Scent leaf)</td>
<td>1.77±0.12\textsuperscript{d}</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD. n=4. Values on the same column with different superscripts differ significantly (p < 0.05)

Located at the lower back of the brain beneath the occipital lobes, the cerebellum coordinates body and voluntary movements by fine-tuning commands from the motor cortex in the cerebrum. The cerebellum also maintains posture and balance by controlling muscle tone and sensing the position of the limbs. All motor activity, from hitting a baseball to fingerling a violin, depends on the cerebellum.

#### Table 3. Cerebellar changes in SOD level for *O. gratissimum* fed, lead II acetate exposed rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (µ/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Control)</td>
<td>77.04±2.08\textsuperscript{a}</td>
</tr>
<tr>
<td>2 (Scent Leaf)</td>
<td>80.39±3.02\textsuperscript{a}</td>
</tr>
<tr>
<td>3 (Lead Acetate)</td>
<td>44.24±1.87\textsuperscript{a}</td>
</tr>
<tr>
<td>4 (Lead acetate + low dose Scent leaf)</td>
<td>48.09±2.74\textsuperscript{a}</td>
</tr>
<tr>
<td>5 (Lead acetate + high dose Scent leaf)</td>
<td>58.01±4.05\textsuperscript{a}</td>
</tr>
</tbody>
</table>

Values are presented as Mean ± SD. n=4. Values on the same column with different superscripts differ significantly (p < 0.05)

#### Table 4. Cerebellar changes in catalase level for *O. gratissimum* fed, lead II acetate exposed rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Catalase (µ/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Control)</td>
<td>2.74±0.05\textsuperscript{a}</td>
</tr>
<tr>
<td>2 (Scent Leaf)</td>
<td>3.22±0.03\textsuperscript{b}</td>
</tr>
<tr>
<td>3 (Lead Acetate)</td>
<td>0.81±0.09\textsuperscript{c}</td>
</tr>
<tr>
<td>4 (Lead acetate + low dose Scent leaf)</td>
<td>1.30±0.12\textsuperscript{d}</td>
</tr>
<tr>
<td>5 (Lead acetate + high dose Scent leaf)</td>
<td>1.46±0.01\textsuperscript{d}</td>
</tr>
</tbody>
</table>

Values are presented as Mean ± SD. n=4. Values on the same column with different superscripts differ significantly (p < 0.05)
4.1 Effects *O. gratissimum* on Oxidative Stress Markers

Tables 1 – 4 summarized the cerebellar levels of oxidative stress markers due to *O. gratissimum* administration. Here, GSH levels of lead II acetate exposed rats (Table 1, group III) had a significantly decreased value for cerebellum upon comparison with control (group 1).

![Fig. 1. Microscopic session of the cerebellum of group I (Control group) rats](image1)

Above figure shows an *H* and *E* stained section of the cerebellum for control group. *Mg: X 100 and X 400*

![Fig. 2. Microscopic session of the cerebellum of group II rats](image2)

Above figure shows an *H* and *E* stained section of the cerebellum for group II animals. *Mg: X 100 and X 400*

![Fig. 3. Microscopic session of the cerebellum of group III rats](image3)

Above figure shows an *H* and *E* stained section of the cerebellum for group III animals. *Mg: X 100 and X 400*

**Table 5. Stereological count of Cerebellar cells in *O. gratissimum* administered Lead II acetate exposed rats**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 (Control)</th>
<th>Group 2 35 days (O.G)</th>
<th>Group 3 21 days (L.A)</th>
<th>Group 4 35 days (L.A and O.G)</th>
<th>Group 5 35 days of (L.A and O.G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebellum Purkinje (Cells)</td>
<td>2.91±1.58^a</td>
<td>2.47±1.33^a</td>
<td>1.83±1.03^b</td>
<td>2.39±0.98^a</td>
<td>2.53±1.21^b</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD. Values followed by different alphabet superscripts indicate a significant difference. O.G = *Ocimum gratissimum* L.A = Lead II acetate
This suggests that dose-dependent treatment with *O. gratissimum* extract caused a significant improvement in GSH levels at all cost in the cerebellum of group III rats, implying minimal, but improved rejuvenation of the neuroglia cells within this area (cerebellum). For group II rats (scent leaf treated), higher regeneration was seen upon comparison with control. In addition, while group (group III) had the lowest mean value of regenerated neuroglia cells (as seen in GSH level) after treatment with *O. gratissimum* extract, groups V, IV and II animals followed respectively with regeneration rates. This can best be explained from the clearance of free radicals by test substance following prolonged feeding with lead acetate. This is because; lead acetate is a known poisonous compound that can alter morpho-functional and biochemical activities via free radical production. This finding concurs with that of Harrison and Eastwood, 2001 [20].

From Table 2, rats treated with test extract (Group 2) had the significantly lowest MDA values than those of control and other groups. Contrarily, Group III rats showed a significantly increased mean MDA value (p < 0.05) upon comparison with control (Group I) and other groups. Thus, it can be inferred that, following period of rats’ exposure to lead II acetate, treatment with *O. gratissimum* extract caused a significant decrease (p < 0.05) in MDA levels of the cerebellum for all groups, except group III when compared with control. This happened though, not to a level comparable with those in other groups.

Physiologically, this explains that high and low dose treatment with *O. gratissimum* significantly improves cerebellar MDA activities, aligning with previous reports of Timmann and Daum, who found elevated levels of lead (Pb2+) to aggravate through different brain targets; oxidative stressors, as well as cognitive and behavioural deficits by inhibiting the N-methyl-D-aspartate receptor (NMDAR), resulting in a significant reduction of Ca2+ influx into the cerebrum, plus alteration of metabolic pathways that upregulates synaptic development and neurotransmissions in brain [21].

Table 3 shows results of average Superoxide Dismutase (SOD) levels for *O. gratissimum* extract treated rats across groups. Here, highest SOD levels were decreasingly seen in groups II, V, IV and II respectively upon comparison with
4.2 Possible Mechanism of Action of *O. gratissimum* against Oxidative Stressors in Examined Tissues

Oxidative stress is defined by the balance between the generation of Reactive Oxygen Species (ROS) such as super-oxide anion and the antioxidant defence systems such as superoxide dismutase (SOD). Antioxidant enzymes are involved in the elimination of ROS including SOD, CAT and GSH [19].

Data from present study showed a decrease in some activities of all measured antioxidants enzymes in rats, which are clinically useful in assaying for oxidative stress; in this case, indicative of decreased oxidative stress in concerned tissue. This will also be suggestive of a decrease in the anti-oxidant defence system that ordinarily could be potent in regeneration of damaged cerebellar tissues following duration-dependent administration of lead II acetate. However, treatment with *O. gratissimum* in any of such concerned group of rats, very minimally increased the activities of the anti-oxidant enzymes in some cases. Since oxidative stress contributes meaningfully to the pathophysiology of brain damage, substances that overwhelm oxidative stress might be therapeutically beneficial [18-19]. From outcome of Tables 1 through 4 *O. gratissimum* seems to be a potential example (anti-oxidant) as such in any case of increased oxidative stressors (though regeneration was minimal) for test tissues.

From this study, though underlying mechanism(s) of regeneration of neuroglia cells following duration and dose dependent administration of *O. gratissimum* extract on rats exposed to lead acetate may not be fully unravelled; Anti-oxidative interplays are however suspected. By this, intracellular enzymes such as Superoxide dismutase (SOD) and Catalase along with non-enzymatic antioxidants (glutathione -GSH) are known to act as primary line of defence to cope with deleterious effects of reactive oxygen species that may have result from damaged tissues, following lead acetate administration. SOD purifies superoxide radicals, converting them to hydrogen peroxide which is then further converted to water by Catalase (CAT) and Glutathione Peroxidase (GPx). Reduced removal of free radicals by SOD, decreased Gluthathione and decreased activities of Catalase are related to anti-aging and anti-degenerative tendencies in tissue and vascular pathology. This reducing capacity of antioxidant enzymes have been reported to lead to decrease oxidative stress in damaged or damaging tissues [18].

Figs. 1 through 5 shows a histological session through the cerebellum of rats for each of the test group at different magnifications with H & E stains. Here, A section of the cerebellum in Group 1 (Control group) showed the cerebellar cortex to consist of molecular layer (ML), granular layer (GL) and Purkinje layer (PL) with an outer cortex of grey matter and a central core of white matter (WM). Cellular features showed the outer molecular layer as consisting of neuronal cell bodies with prominent nucleoli. Within the Purkinje cell layer are purkinje neurons with relatively large cell bodies and centrally placed round to oval nucleus. The granular layer was also observed to be extremely cellular, composing of numerous and deeply stained basophilic cell bodies that were nodular. Cell membrane of the three layers of the cerebellum appeared indistinct.

5. CONCLUSION

Glosis and regeneration of damaged cerebellar glia cells have been seen in this study. Study has also shown that *O. gratissimum* extract consumption is potent for low to minimal anti-oxidant effect on the cerebellum.
6. RECOMMENDATIONS

We recommend further and highly molecular approach to this study. Extension of similar study to other areas of the brain aside the cerebellum.

CONSENT

Not applicable.

ETHICAL APPROVAL

Ethical clearance was obtained from the Bioethics and Research Committee of the Faculty of Basic Medical Sciences, Delta State University. This was conveyed through a letter as recommended by the International Society for Applied Ethology on the use and care of animals for research.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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