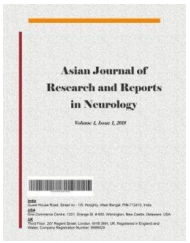

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Lead II Acetate Induced Physio-Morphological Changes in Prefrontal Cortex of *Ocimum gratissimum* Fed Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Indiscriminate consumption of herbs or medicinal plants is said to be harmful without caution. Overtime, the effects of consumption of many of these plants on the brain have remained mysterious. *Ocimum gratissimum* (scent leaf) is one of such numerous herbs commonly used in Nigeria. This study was undertaken to determine the activity of *Ocimum gratissimum* leaf extract on the prefrontal cortical area of lead II acetate exposed wistar rats. Twenty five (25) Wistar rats of five (5) rats each were divided into five (5) groups. With Group one (1) being control group (fed with standard rat diet and water only), Groups 2, 3, 4 and 5 formed the experimental group and respectively received 252.98 mg/kg of body weight (bwt)/day of aqueous *Ocimum 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 bwt of *Ocimum Gratissimum* leaves extract for 35 days, and 180 mg/kg of lead II acetate for 21 days with continued treatment of 252.98 mg/kg bwt of

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Ocimum Gratissimum leaves extract for 35 days. Following treatment period, rats were euthanized and blood samples assayed for biochemical changes. Histological and stereological examinations were also conducted for harvested brain tissue; the prefrontal cortex. Upon comparison with control (group I), a significant decrease was seen in prefrontal cortical Malonaldehyde, Superoxide Dismutase, Glutathione and Catalase levels, suggestive of a relatively fair regeneration of damaged glia cells (Gliosis) across groups. Again, histological section revealed the presence of pyramidal neurons with polygonal cell bodies of prominent dispersed nucleoli, containing chromatin granules with glial cells; Indicative of a potential possibility for gliosis. Similar studies on other areas of the brain are highly recommended.

Keywords: Ocimum gratissimum; prefrontal cortex; oxidative stressors.

1. INTRODUCTION

As an alternative to orthodox therapy, medicinal plants are often commonly used by the inhabitants of developing countries. In Africa alone, hundreds of plants are used traditionally for the management and/or control of different ailments. Regrettably, only a few of such plants have received scientific examination [1].

In several countries, *Ocimum gratissimum* has been used extensively in the traditional system of medicine. In the North east of Brazil for instance, it is used for medicinal, condiment and culinary purpose. The flowers and the leaves of this plant are rich in essential oils so it is 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 [4]. *O. gratissimum* is used by the Ibos 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 [5].

As controversies on consumption of *O. Gratissimum* rages, divergent views have found it to produce negative (undesirable) and/or positive (desirable) effects. Regarding conflicting outcomes of epidemiological studies on *O. Gratissimum* and its effects on reproductive indices, *O. Gratissimum*-containing foods are gradually becoming one of the most consumed by the human populations of the world. Its health effects have been, and are still being extensively investigated, especially in Africa [6]. *O. Gratissimum* is known to pose some beneficial antioxidant effects as a result of its component ingredients; Alkaloids, Tannin, Saponin and Flavonoids [6-7]. Being one of the World's most widely consumed herbs, it is legal and unregulated in nearly all jurisdictions [7].

Currently, an estimated 15% of the world's population consumes O. *gratissimum*-containing substance daily [8]. Given this widespread use, its potential health effects are important for public health as well as for helping individuals make informed choice regarding its consumption. Empirically, though little or no works exist on the activity of *O. gratissimum* on the brain, however, studies to investigate whether seasonal variations in composition of essential oil of *O. gratissimum* are accompanied by changes in pharmacological properties have report its potency in increasing barbiturate-induced sleep duration in the brain [9-10]. In situations of Lead poisoning, *O. gratissimum* has been reported to be a potent adsorbent that helps in leveraging most anticipated resultant complications [11].

Lead poisoning, which has been shown to affect numerous organ systems, is associated with a number of morphological, biochemical and physiological changes, including kidney dysfunction, abnormal glucose metabolism, nervous system disturbances, impairment of liver function and haematological disorders [11]. Lead poisoning shows verity of symptoms and signs which varies according to the individual and duration of exposure of the individual to lead [12]. The toxic effect of lead depends whether the agent is an organic compound "one that contains carbon" or inorganic compound [13]. Lead has been associated with various kinds of cancer, nephrotoxicity, neurotoxicity and cardiovascular diseases in Man [12]. The toxicity of lead (Pb) is closely related to age, sex, route of exposure, level of intake, solubility, metal oxidation state, retention percentage, frequency of intake, absorption rate, duration of exposure, mechanisms and efficiency of excretion. Moreover, excess lead is known to reduce the cognitive development and intellectual performance in children [13].

In the light of this, the present study was therefore undertaken to determine the effect of *O. gratissimum* on the brain of lead II acetate induced adult male wistar rats. The histology of the prefrontal cortex was evaluated. Biomarkers of oxidative stress (Catalase, Malonaldehyde, Glutathione and Superoxide Dismutase) were also assayed with stereological analysis as accomplice.

1.1 Aim of Study

Study investigated the morphological, biochemical and functional changes in the prefrontal cortex of the brain; following duration and dose-dependent administration of aqueous extract of *O. gratissimum* leaves to lead II acetate induced wistar rats. Specifically, study;

i. Evaluated the anti-oxidant effect of *O. gratissimum* on the prefrontal cortex of lead II acetate exposed adult Wistar rats.
ii. Investigated the ameliorating effect of *O. gratissimum* on the histo-architecture of the prefrontal cortex of adult Wistar rats induced with lead II acetate.

2. MATERIALS AND METHODS

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 study's sensitivity and invasive nature, Wistar rats were chosen as experimental model.

2.2 Study Design

Study was an experimental type of research design, and comprised of twenty five (25) wistar rats; which were purchased from the animal house of the Faculty of Basic Medical Sciences, Delta State University, Abraka and housed in wooden cages for a two (II) weeks period to allow for acclimatization; following which animals were divided into five (V) groups; each consisting of five (V) rats per group. Group one (I) was tagged control group, and received standard rat feeds and water only ad libitum. Groups II, III, IV and V formed the treatment groups with each receiving 252.98 mg/kg of body weight (bwt)/day of aqueous *Ocimum 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 bwt of *Ocimum gratissimum* leaves extract for 35 days, and 180 mg/kg of lead II acetate for 21 days with continued treatment of 252.98 mg/kg bwt of *Ocimum gratissimum* leaves extract for 35 days respectively.

2.3 Resources and Sources

2.3.1 Plant sample collection and identification

O. gratissimum (scent) Fresh leaves were obtained from the premises of site three commonly called campus three of Delta State University, Abraka. The scent leaves was identified and authenticated by certified and experienced taxonomists from the Department of Botany, Delta State University, Abraka.

2.3.2 Preparation of plant's extract

Obtained *O. gratissimum* (scent) leaves was extracted in the department of pharmacology, Delta State University, Abraka. The fresh scent leaves were air-dried in the laboratory at ambient temperature (30 ± 2°C) for 10 days. They were thereafter pulverized with a laboratory mechanical grinder. The obtained fine powders were then stored until needed. 50g of the powdered sample was extracted with distilled water of 500mls (via maceration) for 48hrs. The mixture was then decanted and filtered with sterile whatman paper. Next, weighed samples (20 g in 10 ml distilled water) of the extract were used to prepare the stock solution (500 mg/ml) as has been previously described by Eno et al. [14]. The stock solution was then labelled appropriately and refrigerated at $4C^{O}$ until required for use.

2.3.3 Acute toxicity (LD_{50}) determination

As recommended by Mohammed et al. [14], LD_{50} for oral administration of aqueous *O. gratissimum* leave extract on Wistar rats is 1264.9 mg/kg, with safe dose being 1/10 of LD_{50} as recommended by Neharkar and Gaikward. [16]. 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, LD_{50} for its oral administration to wistar rats as recommended by Neharkar and Gaikward. [16] is 600 mg/kg, with safe dose being 1/10 of LD_{50}. This study used a 30% (180 mg) dose for lead II acetate on experimental rats.

2.4 Ethical Considerations

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

2.5 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 8:00am and 10:00am daily as recommended by Ejebe et al. [14].

2.6 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.

2.7 Sample Collection

At the end of the entire process, a 5 ml syringe, blood samples were collected from inferior vena cavae of animals, following cervical dislocation; and emptied into a well calibrated bottle. In any case, obtained blood samples were centrifuged at a resolution of 300 rpm for 10 minutes. Cleared supernatants were aspirated into plain bottles and refrigerated at 4°C before biochemical analysis. Prefrontal Cortical tissues were thereafter harvested and stored in a container of 10% formol-saline for fixation and histological photomicrography.

2.8 Histological Procedure

2.8.1 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.

2.8.2 Sectioning and mounting

At 5 microns (μ), the blocks of tissues were sectioned with a rotary microtome. 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 polystrene), 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.

2.9 Biochemical Estimations

To ascertain the level of oxidative damage and rejuvenation, the Prefrontal cortex was subjected to the following biochemical estimations.

1. **Super oxide dismutase:** Which was basically accessed through the activity of acetyl cholinesterase enzyme as recommended by Kono [15]. Outcome was expressed in units/mg.
2. **Catalase:** Whose activity was determined in accordance with the methods of Luck [16].
3. **Glutathione (GSH):** Glutathione activity was determined by the method of Moron et al. [17]. Here, enzyme activity was expressed in l mol of GSH/g of assayed tissue.
4. **Malondialdehyde (MDA):** The activity of Malondialdehyde was determined by assaying for the quantity of the Thio-Barbituric Acid (TBARs)

2.10 Analytical Approach

Stained slides were interpreted with the aid of a light microscope and analyzed for both

histological and morphological changes across the groups. Statistical significance for obtained outcome was analyzed with the one-way analysis of variance (ANOVA). All of these processes were automated and achieved through statistical package for social sciences (SPSS) version 20. Differences between means were considered at $p < 0.05$.

3. RESULTS

Refer to below tables and figures for collated results following careful statistical analysis.

Table 1. Effect of *O. Gratissimum* on GSH level of prefrontal cortex of Lead II acetate exposed rats

Groups	Prefrontal cortex
1 (Control)	43.8±1.28[a]
2 (Scent Leaf)	48.8±1.30[a]
3 (Lead Acetate)	28.4±1.22[b]
4 (Lead acetate + low dose Scent leaf)	36.7±2.09[c]
5 (Lead acetate + high dose Scent leaf)	39.3±1.36[c]

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

Table 2. Changes in MDA levels of prefrontal cortex *in O. Gratissimum* administered Lead II acetate exposed rats

Groups	Prefrontal Cortex
1 (Control)	2.54±0.02[a]
2 (Scent Leaf)	2.02±0.05[b]
3 (Lead Acetate)	5.69±0.03[c]
4 (Lead acetate + low dose Scent leaf)	4.21±0.07[d]
5 (Lead acetate + high dose Scent leaf)	4.48±0.09[d]

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

Table 3. Changes in SOD levels of prefrontal cortex *in O. Gratissimum* administered Lead II acetate exposed rats

Groups	Prefrontal Cortex
1 (Control)	44.03±1.29[a]
2 (Scent Leaf)	59.05±2.03[b]
3 (Lead Acetate)	27.14±2.01[c]
4 (Lead acetate + low dose Scent leaf)	35.11±3.89[d]
5 (Lead acetate + high dose Scent leaf)	38.02±3.56[e]

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

Table 4. Changes in catalase levels of prefrontal cortex *in O. Gratissimum* administered Lead II acetate exposed rats

Groups	Prefrontal Cortex
1 (Control)	1.85±0.07[a]
2 (Scent Leaf)	2.18±0.01[b]
3 (Lead Acetate)	0.47±0.02[c]
4 (Lead acetate + low dose Scent leaf)	0.81±0.03[d]
5 (Lead acetate + high dose Scent leaf)	0.87±0.05[e]

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

Table 5. Stereological count of prefrontal pyramidal cells *in O. Gratissimum* administered Lead II acetate exposed rats

Parameter	Group 1 (Control)	Group 2 35 days (O.G)	Group 3 21 days (L .A)	Group 4 35 days (L.A and O.G)	Group 5 35 days of (L.A and O.G)
Prefrontal cortex (pyramidal cells)	9.91±3.40[a]	8.48±3.03[b]	5.20±2.10[c]	9.83±2.84[a]	7.18±2.91[d]

All 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

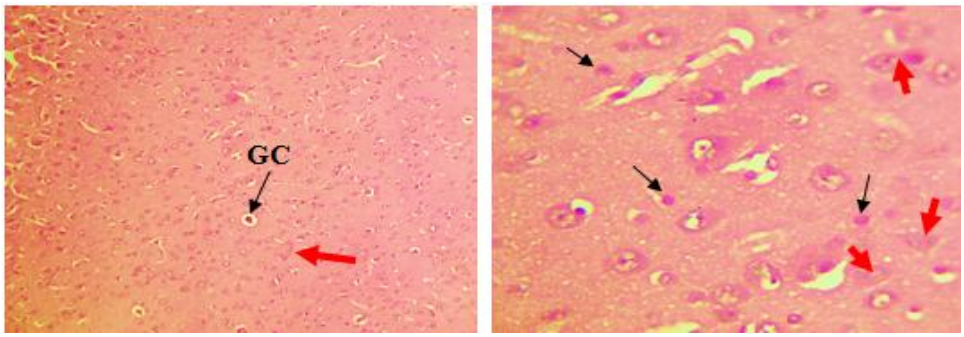

Fig. 1. H & E stained section through the prefrontal cortex of Group 1 (Control group)
Above shows normal orientation of the prefrontal cortex with some layers of the cerebral cortex Mg: X 100 and X 400

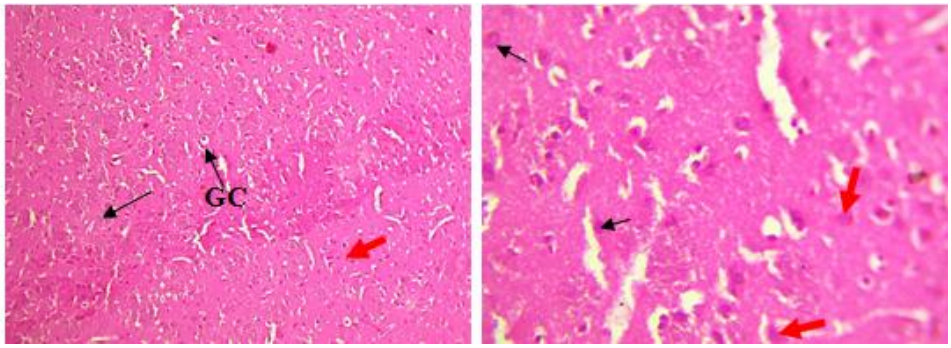

Fig.2. H & E stained section of the prefrontal cortex for Group II rats
Mg: X 100 and X 400

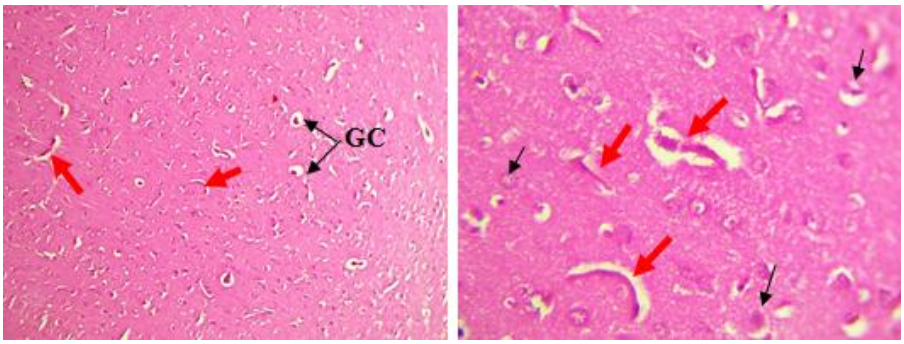

Fig. 3. H & E stained section of the prefrontal cortex for Group III rats
Mg: X 100 and X 400

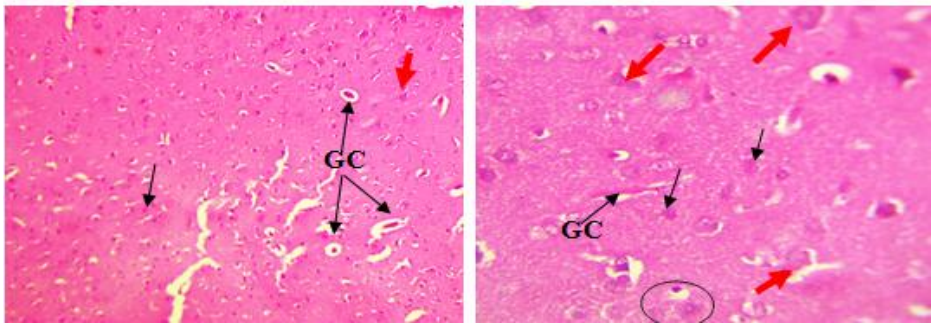

Fig. 4. H & E stained section of the prefrontal cortex for Group IV rats
Mg: X 100 and X 400

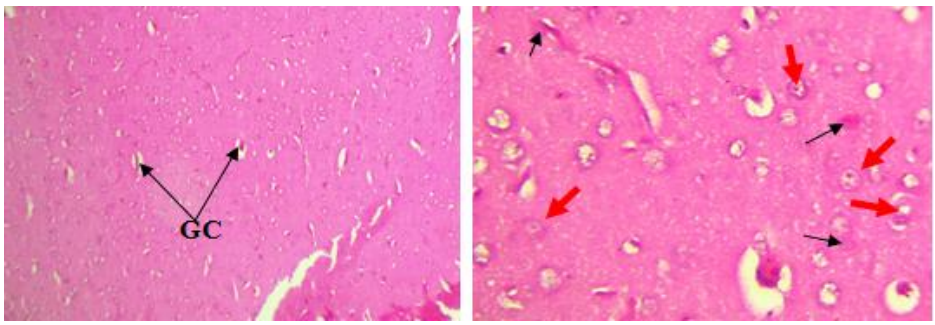

Fig. 5. H & E stained section of the prefrontal cortex for Group V rats
Mg: X 100 and X 400

4. DISCUSSION

O. Gratissimum has been medically applied as antimalarial, anticonvulsant, antibiotics, antifungal, anti-epilepsy, and in mental illnesses [18]. In some part of Niger Delta (Nigeria), it is also used as anti-diarrhoea. Even though little has been done on its effect on the brain [19], current study exermined the activity of *O. Gratissimum* leaf extract on the prefrontal cortex of the brain.

4.1 Changes in Oxidative Stress Markers with *O. gratissimum* Administration

Tables 1 – 4 summarize the effect of *Ocimum gratissimum* on biomarkers of oxidative stress. For the prefrontal GSH level of rats exposed to lead II acetate, result (Table 1) shows a significantly lower value of GSH in the pre-frontal cortex as compared with control (Group 1).

This implies that treatment with *O. Gratissimum* extract at all doses significantly improved GSH levels in the prefrontal cortex of control (group 1) rats, suggestive that regeneration of neuroglia cells in this area (prefrontal cortex) was relatively improved (though minimal). For group II animals (scent leaf treated), a higher regeneration was seen as compared to those in group 1. again, whereas, lead Acetate treated group (group III) had a high level of degeneration of neuroglia cells due to high level of GSH, very little or no regenerative outcome due to GSH was visible for groups IV (Lead Acetate + low dose scent leaf) and V (Lead Acetate + high dose scent leaf) rats. The reason for high level of GSH with little or no regeneration in group III rats is not far-fetched, as lead poisoning may have affected numerous morpho-functional and biochemical processes ex-vivo. This finding firmly agrees with that of Harrison and Eastwood, 2001 who observed that complex cerebral processes may be altered following dose-dependent administration of lead with minimal or no attenuation rates [20].

Table 2 shows the Effect of *O. Gratissimum* on the level of MDA level of the prefrontal cortex of lead II acetate exposed rats. From the table, rats treated with *O. Gratissimum* leaf extract (Group 2) significantly showed lower MDA level than those of the control group. Contrary to this, rats exposed to lead II acetate alone (Group III) had a significantly increased levels of MDA ($p < 0.05$) compared to control (Group I). The treatment of lead acetate-exposed rats with high and low

doses of *O. Gratissimum* extract (Groups 4 and 5) caused a significant decrease ($p < 0.05$) in MDA level compared to rats maintained on lead II acetate alone (Group 3), though not to a level comparable with those in control group.

Result from Table 2 also showed a significantly decreased MDA level in the prefrontal cortex for all treated groups except in lead II acetate treated group (Group III) when compared with control. However, when the prefrontal regions in groups IV (Lead acetate + low dose Scent leaf) and V (Lead acetate + high dose Scent leaf) rats were compared with control, there was a significant increase in MDA levels across groups. This physiologically implies that treatment with *O. Gratissimum* at high and low doses, significantly improves MDA activities in the prefrontal cortex. This finding strongly allies with those of Timmann and Daum, who found that, Elevated levels of lead (Pb2+) can induce through oxidative stressors, cognitive and behavioral deficits in adults and children with different brain targets by inhibiting the N-methyl-D-aspartate receptor (NMDAR). Inhibition of NMDAR result in a significant reduction of Ca^{2+} entry into the cerebral cells, altering metabolic pathways involved in synaptic development and neurotransmissions in brain [21].

For superoxide dismutase (SOD), results from Table 3 proved that rats treated with extract of *O. Gratissimum* only (Group II) had a significantly higher SOD level than to control in all examined tissues. Conversely, exposure to lead acetate alone (Group III) significantly reduced SOD ($p < 0.05$) compared to control (Group I), maintaining a higher and lower SOD values for the prefrontal cortex of groups IV and V respectively as compared with control. This treatment with high and low doses of *O. Gratissimum* extract (Groups 4 and 5) brought about a significant increase ($p < 0.05$) in SOD level compared to rats maintained on lead II acetate alone (Group III), following comparisons with those exclusively fed with *O. Gratissimum* extract. Howbeit, this was however attenuated in high and low doses of *O. Gratissimum* as amelioration was seen in SOD level.

Table 4 shows the effect of *Ocimum gratissimum* leaf extract on catalase activity in prefrontal cortex of lead II acetate-exposed rats. From the table, a significant increase is seen in catalase activities across all examined groups in comparison with control; even though subsequent exposure to lead acetate II alone (in group III) caused a significant reduction ($p < 0.05$) in catalase activity upon comparison with control (Group I). Similar findings were observed in other enzymes involved in antioxidant defence systems, implying that treatment with *O. Gratissimum* at all doses significantly influenced catalase-mediated oxidative stress on the assayed tissues [22].

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 for regeneration of damaged prefrontal 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 the outcome of tables 1 through Table 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 a 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 Glutathione and decreased activities of Catalase are related to anti-ageing and anti-degenerative tendencies in tissue and vascular pathology. This reducing capacity of antioxidant enzymes have been reported to lead to decreasing oxidative stress in damaged or damaging tissues [18].

4.3 Benefit of Study

Banning the use of lead in petrol additive has reduced human toxicity but its exposure is still a separate public health issue worldwide. This study will, therefore, be relevant to victims of inadvertent exposure to lead II acetate in providing a quick, accessible and herbal alternative treatment option in any of such cases that may result in brain damage. Findings from this study will also add to health professionals' knowledge about lead induced toxicity on the brain.

5. CONCLUSION

Findings from this study has shown that consumption of *O. gratissimum* leaf extract has low to minimal anti-oxidant effect on degenerating brain cells. A great deal of gliosis and regeneration of damaged glia cells were also visible. Implicating *O. gratissimum extract as* as potent positive attenuator of the histo-architectural framework of a degenerating prefrontal cortical tissue.

6. RECOMMENDATIONS

We recommend for more sophisticated and highly molecular approach to this study. Extension of similar study to other areas of the brain (besides prefrontal cortex) is also highly recommended.

CONSENT

It is not applicable.

ETHICAL CONSIDERATIONS

Approval for this study was obtained from the Bioethics and Research Committee of the Faculty of Basic Medical Sciences, Delta State University which 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

Authors have declared that no competing interests exist.

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