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NEUROPROTECTIVE EFFECTS OF THE AQUEOUS ROOT EXTRACTS OF *CURCUMA LONGA* (TURMERIC) ON THE HISTOLOGY OF THE HIPPOCAMPUS IN RAT MODELS OF LEAD ACETAT-INDUCED NEUROTOXICITY

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Abstract: **Background: Lead is a neurotoxic substance and its exposure has been recorded to impair memory and learning. The hippocampus of the brain is vital to learning and memory consolidation. *Curcuma longa* (Turmeric) is a medicinal plant with bioactive compounds, utilized in traditional medicine especially for neurological disorders. Aim: This study aims at discovering the neuroprotective effects of the aqueous root extracts of *C.longa* (ARECL) on the histology of the hippocampus after lead-acetate induced neurotoxicity. Methodology: 30 non-pregnant adult female wistar rats (150-200g) were divided into 6 groups of 5 rats each. Group A (control) received *ad libitum*. Group B (Untreated) received 30mg/kg of lead acetate daily. Group C-F all received 30mg/kg of lead acetate daily for 12 days and then received simultaneous administration of 30mg/kg of lead acetate and 100, 300, 500mg/kg of the ARECL and 100mg/kg of Vitamin E respectively daily for another 12 days. All administrations were oral and the experiment lasted 24 days. The animals were sacrificed under ketamine as anesthesia, 24 hours after their last treatment. The brain was carefully harvested and the hippocampus isolated, fixed accordingly and processed for routine H&E staining. Results: Untreated group displayed degenerative injuries such as widespread degeneration of the neurons and cytoplasmic vacuolations observed in all distinct layers of the hippocampus relative to the control. Treatment with ARECL demonstrated high levels of neuroprotection as they maintained the normal histo-architecture of the hippocampus. Conclusion: This study provided evidence that *C. longa* exhibits a strong dose-dependent neuroprotective capacity on the histology of the hippocampus after neuro-intoxication with Lead Acetate.**

Keywords: ***Curcuma longa*****, neuro-protection, neuro-intoxication, Lead Acetate.**

1. INTRODUCTION

Increasing industrialization exposes the human population to hazardous environmental pollutants. One of such pollutants of concern which is known to cause tremendous harm to humans is Lead (Pb) (Missoun et al., 2010). Tissue damage caused by lead intoxication is linked to its ability to cause the generation of reactive oxygen species within the tissues resulting to oxidative stress-related injuries (Pajović *et al*., 2003; Sanders *et al*., 2009).

Lead toxicity is one of the common forms of heavy metal intoxication (Kumar and Scott-Clark, 2009) which has been associated to neurotoxicity due to its ability to pass through the blood-brain barrier, causing different neurological disorders such as behavioral problems, nerve damage, mental retardation (Lanphear, 2005). Alzheimer's disease, Parkinson's disease and schizophrenia have also been associated to lead toxicity (Sanders, *et al.*, 2009). The hippocampus plays a very vital role in learning as it is responsible for strengthening Short and long-term memory (Mirzaei *et al*., 2013). Lead toxicity is known to affect the neurogenesis in hippocampus (Rahman et al., 2012).

Natural products from medicinal plants have been a resource for drugs development over the years because of their bioactive phytochemical content (Pradeep and Sudipta 2013). *Curcuma longa* (Turmeric) from the ginger family, Zingiberaceae is utilized in traditional medicine for its possible therapeutic effect and its considerably low toxicity (Huang, *et al.*, 2016). Curcumin, bisdemethoxycurcumin and demethoxycurcumin are active polyphenolic compounds and the most bioactive compounds found in *C.longa*, responsible for its strong antioxidant capability (Tayyem *et al.*, 2006; Madiha et al., 2018). Literatures indicating the therapeutic behavior of turmeric in neurological disorders are currently on the rise (Kulkarni and Dhir, 2010; Kim *et al.*, 2012). However, there is minimal exploration on its effect in heavy metal intoxication.

2. MATERIALS AND METHODS

Procurement and Processing of Plant Materials

Fresh roots of *Curcuma longa* were purchased at Ogbete main market Enugu. The roots were authenticated at the Faculty of Agricultural Science, Enugu State University of Science and Technology. Roots were scrupulously washed under a running tap water, cut into smaller pieces and air dried under shade at room temperature for 14 days. Dried roots were grounded to fine powder and sieved. Aqueous extraction was done according to the methods of Sengupta *et al.*, (2011). The extract was kept in airtight containers and stored in a refrigerator at 4°C until ready to use.

Experimental animals

Thirty (30) non-pregnant adult female wistar rats (average weight = 150g) were used for this study. This study was carried out in the Animal facility of the Enugu State University of Science and Technology College of Medicine, Parklane, Enugu. The animals were housed in netted iron cages, kept in well ventilated breeding rooms, allowed to acclimatize for 14 days and provided easy access to food (standard poultry mesh) and water *ad libitum*. The animals were maintained under standard laboratory conditions and handling was done following the guidelines of the college committee for the purpose of control and supervision of experiments on animals. Thirty adult male wistar rats with average weights of 180-200g were used for this study. This study was carried out in the Animal facility of the Enugu State University of Science and Technology College of Medicine, Parklane, Enugu. There were provided easy access to water and standard livestock pellets (Guinea Feed Nigeria Limited) as food. The animals were kept and maintained under standard laboratory conditions and handling was done following international guidelines on the use of experimental animals. Ethical approval was gotten from the university's ethical clearance committee with the ethical right permission number: ESUCOM/ FBMS/ETR/23/008.

Experimental design

The rats were grouped into 6 (n=5). Group A (control group) received food and water *ad libitum*. Group B (untreated) received 30mg/kg of lead acetate daily for 24 days. This dose was adopted from Saleh and Meligy, (2018). Group C-F all received 30mg/kg of lead acetate daily for 12 days and then received simultaneous administration of 30mg/kg of lead acetate and 100, 300, 500mg/kg of the aqueous root extract of *Curcuma longa* (ARECL) and 100mg/kg of Vitamin E respectively daily for another 12 days. This dose of Vitamin E was also adopted from Saleh and Meligy, (2018). All administrations were done orally and the experiment lasted 24 days.

Histological Study

Animals sacrificed were carried out 24 hours after their last treatment, under ketamine (100mg/ml) as anesthesia. The respective brains were carefully harvested and fixed in labeled containers for 24 hours prior to the isolation of the hippocampus for histological tissue processing using the standard protocols and stained accordingly with hematoxylin and eosin for histological studies. Photomicrographs were taken using Amscope 14MP USB 3.0 digital microscope camera at x100 magnification.

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3. RESULTS

Histological Findings

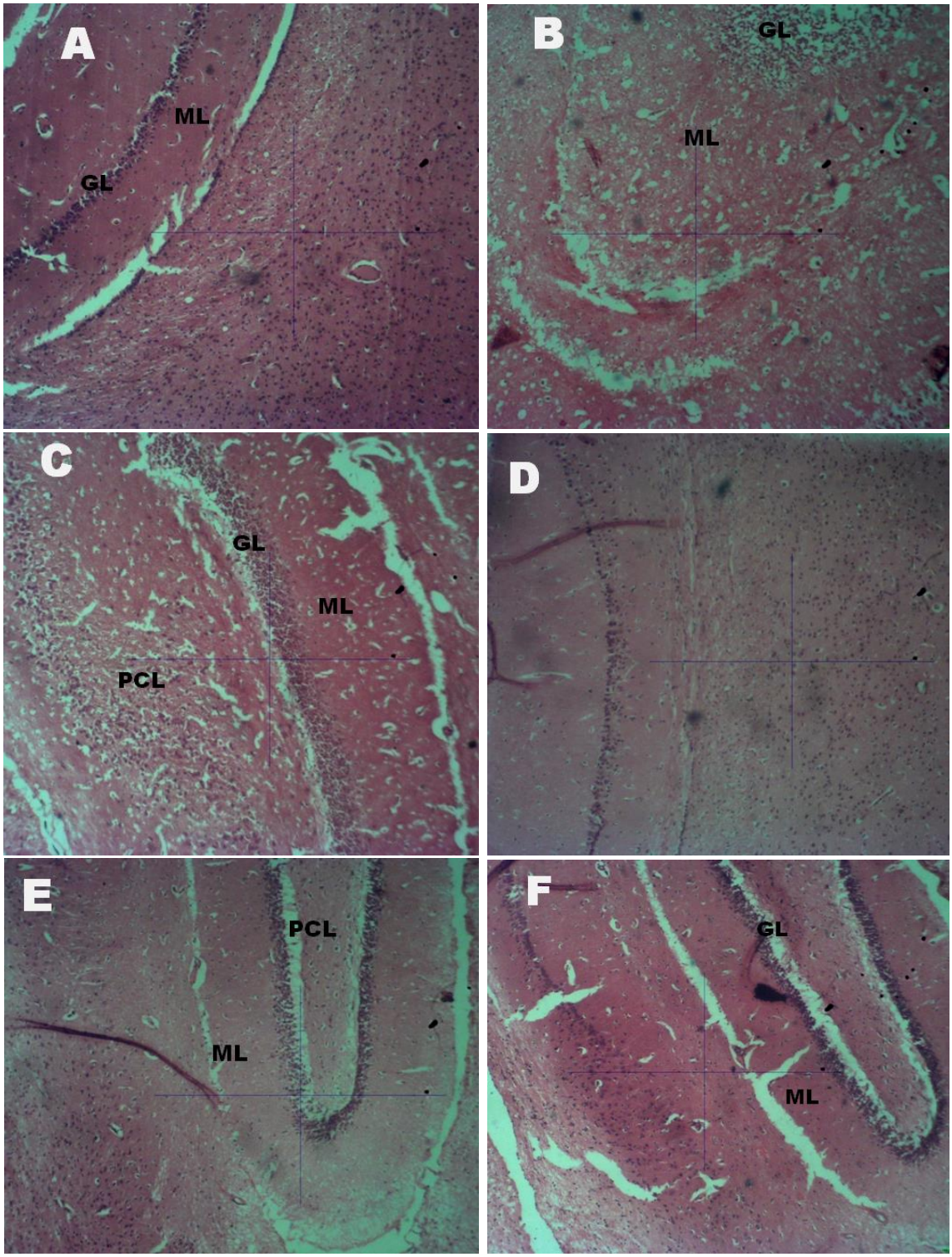

Figure A: (Control group) Photomicrograph of the hippocampus showing the normal histo-architecture of the hippocampal neurons with compact granular cells of the granular layer (GL), large pyramidal cells of the pyramidal cell layer (PCL) and normal glial cells and neurons observed at the molecular layer (ML). **Figure B:** (Untreated group) Hippocampus showing widespread degeneration of the hippocampal neurons with widespread cytoplasmic vacuolations

observed in all layers. **Figure C:** Lead acetate + 100mg/kg of ARECL. Hippocampus showing widespread degeneration of neurons with widespread cytoplasmic vacuolations observed in all layers. **Figure D:** Lead acetate + 300mg/kg of ARECL. Normal histo-architecture of the hippocampal neurons. **Figure E:** Lead acetate + 500mg/kg of ARECL. Normal histo-architecture of the hippocampal neurons. **Figure F:** Lead acetate + 100mg/kg of Vitamin E. Hippocampus showing mild cytoplasmic vacuolations within the hippocampal neurons. H&E. x100.

4. DISCUSSION

Lead is a neurotoxic substance (Lanphear, 2005). This study demonstrated the neurotoxic effects of lead administration on the histology of the hippocampus evident by degenerative injuries (Figure A: widespread degeneration of the neurons and cytoplasmic vacuolations observed in all distinct layers of the hippocampus) relative to the control animal group. These degenerative injuries are suggested to be caused by the increased generation of reactive oxygen species and reduction in the antioxidant activity in the brain tissue caused by the increased concentration of lead within the brain tissue. Previous studies attested that lead intoxication results in oxidative stress-related injuries in body organs and consequent reduction in organ functionality (Pajović *et al*., 2003). Lead intoxication has been recorded to impair memory and learning in male rats (Salehi *et al*., 2015).

Treatment with vitamin E and increasing doses of the aqueous root extracts of *C.longa* (ARECL) displayed different levels of neuroprotection.

Vitamin E displayed moderate neuroprotective potentials evident by the presence of mild degenerative injuries. The neuroprotective effects of vitamin E can be said to be time and dosage- dependent. This is because, previous studies with increased dosage and duration of treatment yielded better results. In a study by Salehi *et al*., (2015), 150mg/kg of vitamin E for 2 months counteracted the harmful effects of Lead intoxication on learning, memory and the histology of the hippocampus in male wistar rats.

Meanwhile, 300 and 500mg/kg of ARECL demonstrated high levels of neuroprotection as they maintained the normal histo-architecture of the hippocampus (Figure D and E) similar to the control group (figure A). This neuroprotective effect can be attributed to the high content of Curcumin and other active polyphenolic compounds found in *C.longa* (Tayyem *et al.*, 2006; Madiha *et al.*, 2018). Our observation also corresponds with previous studies. Kumar *et al.*, (2020) established from his study that curcumin from *C.longa* exhibited strong neuroprotective effects against the progression of fluorosis, particularly in relation to the histology of the hippocampus of the brain, animal behaviour and cognition. Similar observations were demonstrated by Manal *et al.,* (2019), as Ethyl acetate extract of *Curcuma longa* rhizomes gave rise to high concentration of curcuminoids after extraction and exhibited anti-neurodegenerative, anti-oxidant, and anti-apoptotic properties in rats' models Parkinson's disease. Zahra *et al.,* (2012), also reported that daily intraperitoneal injection if 50 mg/kg of Curcumin served as a therapeutic agent for prevention and treatment of homocysteine rat model of Parkinson's disease.

5. CONCLUSION

This study investigated the neuroprotective effects of the aqueous root extracts of *C. longa* (ARECL) on the histology of the hippocampus of wistar rats. This study demonstrates the neurodegenerative effects of Lead (Pb) on the histology of the hippocampus of the brain. It also establishes the fact that *C.longa* exhibited a strong dose-dependent neuroprotective capacity on the histology of the hippocampus after neuro-intoxication with Lead Acetate.

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