

# NEUROPROTECTIVE CAPACITY OF THE ETHANOL LEAF EXTRACTS OF *MORINGA OLEIFERA* ON ALCOHOL-INDUCED CEREBELLAR CORTEX TOXICITY IN ADULT WISTAR RATS

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**Abstract:** Cerebellar degeneration is a common complication of chronic alcoholism. *Moringa oleifera* is naturally occurring medicinal plant frequently used to treat different illnesses especially in the management of neurological disorders. **Aim:** This study aimed to investigate the effects of the ethanol leaf extract of *moringa oleifera* (ELEMO) on the microstructure of the cerebellar cortex in alcohol-induced neurotoxicity. **Methodology:** Twenty (20) adult male wistar rats (150g-200g) were divided into 5 (A-E) (n=4). Group A was the control group and received food and water only. Group B received 2ml of 52.5% v/v aqueous alcohol solution daily. Group C, D and E received simultaneous administrations of 52.5% v/v aqueous alcohol solution and then 100mg/kg, 200mg/kg of the ELEMO and 100mg/kg of Vitamin E respectively daily. All administrations were oral and the experiment lasted 14 days. The animals were sacrificed 24 hours after their last treatment via ketamin (100mg/ml) as anaesthesia. The brain was carefully harvested, washed in normal saline and fixed for 24 hours after which the cerebellum was further harvested, fixed accordingly and processed for routine H&E stainings. **Result:** Alcohol treatment caused neurodegenerative changes in the cerebellar cortex evident by severe depletion of neurons and the presence of gross cytoplasmic vacuolations. 200mg/kg of ELEMO protected the normal Cyto-architectural appearance of the cerebellar cortex relative to the untreated rat group and Vitamin E treated rats. **Conclusion:** *Moringa Oleifera* demonstrated a dose dependant neuroprotective capacity on the histology of the cerebellar cortex from alcohol-induced toxicity. This study substantiates its use in traditional medicine.

**Keywords:** Cerebellar cortex, *Moringa oleifera*, alcohol neurotoxicity, wistar rats.

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## 1. INTRODUCTION

The cerebellum controls body coordination and common signs of heavy alcohol consumption is seen to be a lack of body coordination including a staggering gait (Sullivan et al., 1995). Cerebella damage produces disorders in fine movement, equilibrium, posture, and motor learning (Fine et al., 2002).

Alcoholism is an intricate behavioral disorder that is seen as the inability of a person to control his consumption of alcohol despite being aware of its health, occupational and social consequences (Nutt et al., 2021). The brain is a chief target for the action of alcohol and alcoholism has long been linked to brain damage (Madenn and Andrade, 1997). Alcohol is neurotoxic and directly affects the nerve cells. It is known to lead to problems like Alcohol-related compressive neuropathy, Alcohol-related dementia and Cerebellar degeneration (Nakano et al., 1998; Planas-Ballvé et al., 2017).

*Moringa oleifera* is an economically important naturally occurring plant. Its leaves are frequently used by local traditional healers to treat different illnesses especially in the management of neurological disorders (Bukar *et. al.*, 2010). Methanol extractions from *Moringa oleifera* leaf powder has been reported to possess antioxidant-mediated neuroprotective effects (González-Burgos et al., 2021) and it has also been studied for its antiepileptic, anti-inflammatory and antihypertensive capabilities (Ogbe and Afikku, 2011; Ekong et al., 2017).

## 2. MATERIALS AND METHODS

### Plant Materials and Processing

Fresh leaves of *Moringa oleifera* were obtained from a farmland within Enugu metropolis of Enugu State and were authenticated at the Faculty of Agricultural Science, Enugu State University of Science and Technology. The leaves were washed, left to air-dry under shade for 14 days and afterwards, pulverized to fine powder. The properly sieved fine powder was put into an air tight container and 2.7 liters of analytical ethanol was added and stirred for 2 hours and then allowed to stand for 48 hours. Afterwards, the mixture was sieved with a muslin cloth and further filtered with whatman's filter paper size No.1 to obtain a clear filtrate of the extract. The filtrate was concentrated in a hot water bottle at the temperature of 50°C to remove the ethanol and get a crude concentrate of the plant. The extract was kept in an airtight container and stored in a refrigerator at 4°C until ready to use.

### Alcohol preparation

Pharmaceutical ethanol was purchased from a reputable pharmaceutical store at Ogbete main market Enugu, Enugu State. It was diluted to give a concentration of 52.5% v/v aqueous alcohol solution.

### Experimental animals and Design

This study was carried out in the Animal facility of the Enugu State University of Science and Technology College of Medicine, Parklane, Enugu. 20 healthy adult male wistar rats (average weight = 150g-200g) were maintained under standard laboratory conditions and provided easy access to food (standard poultry mesh) and water. Handling and experimentation was carried out following the guidelines of the college committee for the purpose of control and supervision of experiments on animals.

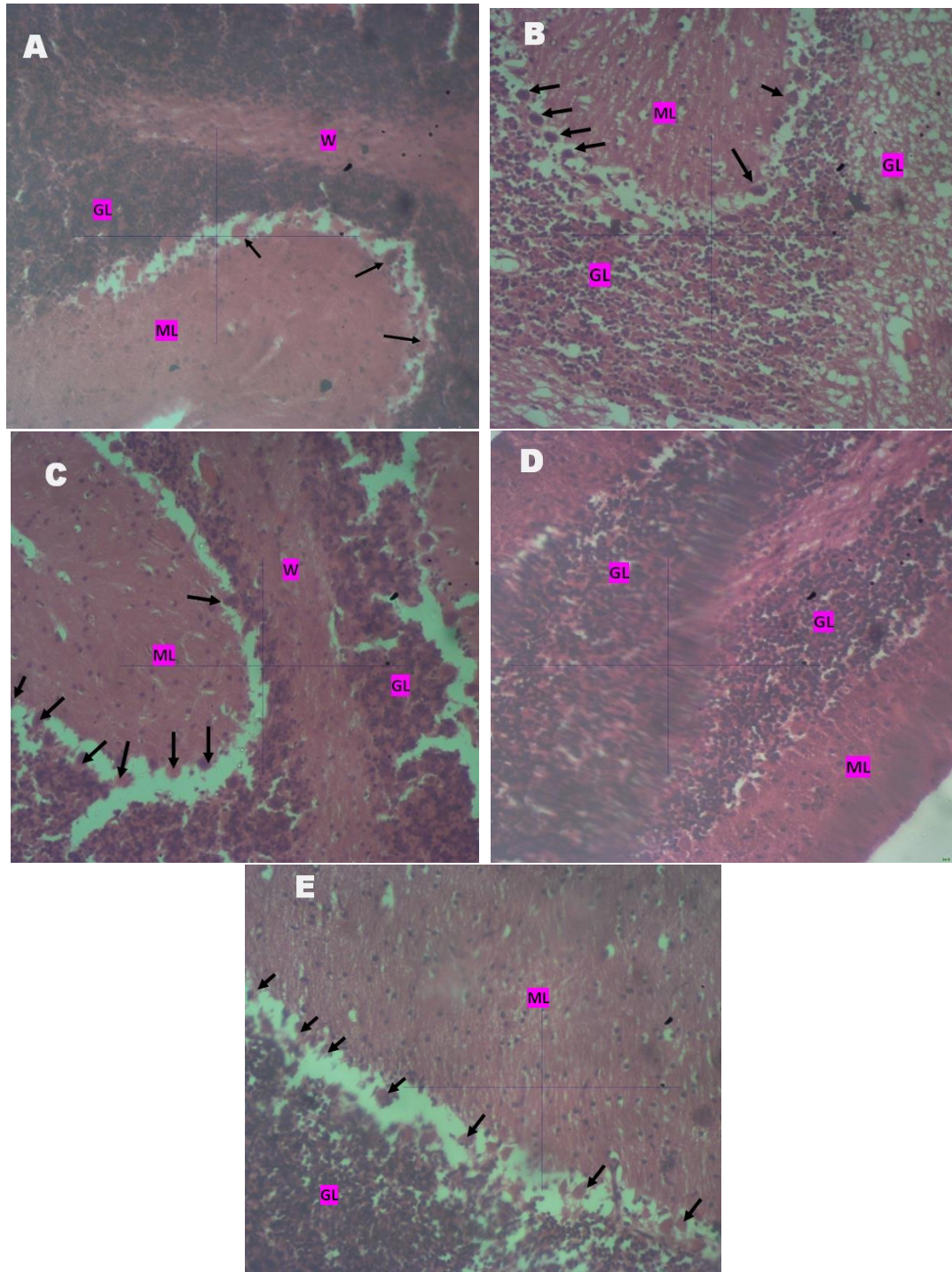
The rats were randomly divided into 5 groups (A-E) (n=4). Group A (control group) received food and water *ad libitum*. Group B (untreated positive control group) received 2ml of 52.5% v/v aqueous alcohol solution daily. This dosage was adopted from Olawale et al., (2018). Group C and D received simultaneous administration of 52.5% v/v aqueous alcohol solution and then 100mg/kg and 200mg/kg of the ethanol leaf extract of *Moringa Oleifera* (ELEMOMO), respectively daily. The dosage of ELEMOMO was adopted from Abijo et al., (2019). Group E received simultaneous administration of 52.5% v/v aqueous alcohol solution and then 100mg/kg of Vitamin E daily. All treatments were done orally and the experiment lasted 14 days.

### Histological Study

The animals were sacrificed 24 hours after their last administration under ketamine (100mg/ml) as anesthesia. The cerebellum was carefully harvested from the brain mass, washed in normal saline and fixed for 48 hours prior to processing. The fixed tissues were processed using the standard protocols for histological tissue processing and stained with hematoxylin and eosin for histological studies. Photomicrographs were taken using Amscope 14MP USB 3.0 digital microscope camera at x100 magnification.

### 3. RESULTS

#### Histological Findings



**Figure A:** Photomicrograph of the cerebellar cortex showing the molecular layer (ML) and the granular layer (GL) with Purkinje cells (black arrow). The white matter (W) is also seen. Cyto-architecture appears normal. H&E. X100. **Figure B:** Cyto-architecture shows severe depletion of neuronal and neuroglial cells in all layers with gross cytoplasmic vacuolations. A prognosis of cerebellar cortex tissue degeneration. H&E. X100. **Figure C:** Cyto-architecture shows focal infarct in the granular cell layer (GL) and depletion of Purkinje cells. H&E. X100. **Figure D:** Cyto-architecture appears normal. H&E. X100. **Figure E:** Cyto-architecture shows mild cytoplasmic vacuolations in the molecular layer (ML). H&E. X100

#### 4. DISCUSSION

Cerebellar degeneration is a frequent feature noticed among chronic alcoholics (Torvik and Torp, 1986; Victor and Lauren, 1978). Some research have also indicated that alcoholism could have detrimental effects on cerebellar functions such as motor coordination (Sullivan et al., 1995A) and peripheral nerve functioning, including the ability to perceive the position of the body and its parts (Palliyath and Schwartz, 1993). This research was carried out to investigate the effect of the ethanol leaf extract of *Moringa oleifera* (ELEMOMO) on the microstructure of the cerebellar cortex in alcohol-induced neurotoxicity.

Daily intake of 52.5% v/v aqueous ethanol solution for 14 days by the untreated animal group caused neurodegenerative changes in the cerebellar cortex which were evident by the severe depletion of neuronal and neuroglial cells and presence of gross cytoplasmic vacuolations. These neurodegenerative changes in the cerebellar cortex can be linked to the oxidative stress caused by the acute oral alcohol intake as these findings are similar to the findings of Olawale et al., (2018), who reported that oral intake of 2mls of 52.5% aqueous ethanol solution caused degenerative changes in the brain histology of experimental rats.

However, treatment with ELEMOMO demonstrated a dose dependant restorative potential on the histology of the cerebellar cortex after alcohol-induced toxicity. While treatment with 100mg/kg of plant extract still had degenerative injuries relative to the control group, 200mg/kg of ELEMOMO restored the normal Cyto-architectural appearance of the cerebellar cortex relative to the untreated rat group and Vitamin E treated rats. This restorative potency can be linked to the antioxidant and neuroprotective properties of *Moringa oleifera* as it corresponds with previous studies.

Djiogue *et al.*, (2022) recorded that treatment 100, 200, or 400 mg/kg of the aqueous extract of leaves of *M. oleifer* demonstrated neuroprotective, and memory-protective effects in scopolamine-treated rats as it prevented scopolamine-induced hippocampal neuron loss. Some isolated protease inhibitors (proline and alanine) from *M. oleifer* leaves were effectively used to improve the degree of axonal damage and treat degenerating axons in experimental rats with induced spinal cord injury (Singh *et al.*, 2012). Hydroalcohol *M. oleifer* leaf extract have been reported to have therapeutic capabilities and also neuroprotective effects against focal cerebral ischemia (Kirisattayakul *et al.*, 2012).

#### 5. CONCLUSION

The ethanol leaf extract of *Moringa oleifera* demonstrated a dose-dependant neuroprotective capacity on the histology of the cerebellar cortex following alcohol-induced toxicity. This justifies its use in traditional medicine.

#### CONSENT

It is not applicable

#### ETHICAL APPROVAL

Ethical clearance was obtained from the Research and Ethical Clearance Committee, Faculty of Basic Medical Sciences, College of Medicine, Enugu State University of Science and Technology with ethical clearance code; ESUCOM/FBMS/ETR/2021/031.

#### COMPETING INTERESTS

Authors have declared that there are no competing interests.

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