ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 13, Issue, 05, pp. 62621-62623, May, 2023
https://doi.org/10.37118/ijdr.26695.05.2023

RESEARCH ARTICLE OPEN ACCESS

NEUROPROTECTIVE EFFECTS OF THE ETHANOL EXTRACT OF MORINGA OLEIFERA ON ALCOHOL-INDUCED PREFRONTAL CORTEX TOXICITY IN ADULT WISTAR RATS

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ARTICLE INFO

Article History:
Received 08th March, 2023
Received in revised form
17th April, 2023
Accepted 26th April, 2023
Published online 24th May, 2023

KeyWords:
Moringa oleifera, Alcohol Neurotoxicity, Prefrontal Cortex, Wistar Rats.

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ABSTRACT

Background: The prefrontal cortex is an area of the brain in the frontal lobe of both cerebral hemispheres that is responsible for decision making, behavior and emotional control (Miller and Cohen, 2001). It has been noted that the prefrontal cortex undergoes functional and structural changes due to chronic alcohol consumption (Abernathy *et al*., 2010; Brodmann, 1909). *Moringa oleifera* is widely known and classified as a medicinal plant but its effects on prefrontal cortex alcohol toxicity have not been substantiated. **Aim:** This study aims at probing the effects of the ethanol leaf extract of *Moringa oleifera* (ELEMO) on the histology of the prefrontal cortex after alcohol-induced neurotoxicity. **Methodology:** Thirty (30) adult male wistar rats (150g-200g) were divided into 5 (A-E) (n=6). Group A was the control group and received feed and water only. Group B received 2ml of 52.5% v/v aqueous alcohol solution daily. Group C and D received simultaneous administrations of 52.5% v/v aqueous alcohol solution and then 100mg/kg and 200mg/kg of the ELEMO, respectively daily. Group E received simultaneous administration of 52.5% v/v aqueous alcohol solution and then 100mg/kg of Vitamin E 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, fixed accordingly for 72 hours and processed for routine H & E staining. **Result:** The ELEMO displayed dose dependant neuroprotective potentials. Low doses had strong therapeutic activity and protected the prefrontal cortex from histopathological alterations while higher doses are suggested to be toxic and led to mild tissue damage. **Conclusion:** The ELEMO can be used in the management of alcohol-induced neurological disorders. However, caution should be applied while consuming this plant.

Citation*: *Christian Chiemeka Ozor and Kingsley Amobi Ejeh. 2023. "Neuroprotective effects of the ethanol extract of *Moringa oleifera* on alcohol-induced prefrontal cortex toxicity in adult wistar rats". *International Journal of Development Research*, 13, (05), 62621-62623.

INTRODUCTION

Excessive alcohol consumption causes alcohol use disorders and weakens an individual's social, work and family relationships (DSM, 2013). More than 60 alcohol-related diseases have been identified by the World Health Organization's report on excessive alcohol consumption (WHO, 2006). The brain is a major target for the action of alcohol, and heavy alcohol consumption has long been associated with brain damage. Chronic alcohol abusers are at additional risk for brain injury from related causes, such as poor nutrition, liver disease and head trauma (Madenn and Andrade, 1997). Alcohol is neurotoxic and it has direct effects on nerve cells. It is known to lead to problems like Alcohol-related compressive neuropathy, Alcohol-related dementia and Cerebellar degeneration (Nakano *et al*., 1996; Planas-Ballvé *et al.*, 2017).

Brain dysfunction is suggested to persist even after the individual has stopped drinking (Eckardt and martin, 2002). Nevertheless, an individual's susceptibility to Alcohol-induced brain damage is greatly unpredictable and is related to factors, such as gender, genetics, environment and socio-demographics (Hartford *et al*., 1991). *Moringa oleifera* is a monogeneric plant in the family Moringaceae which has long been cultivated and all its parts have been consumed and used for diverse purposes across the tropics (Jahn, 1984). This is because if its remarkable range of nutritional and medicinal values (Bukar*et. al.,* 2010). The composition of the amino acids in the leaf protein is well-balanced (Foild *et al*., 2001; Ogbe and Afikku, 2011). Nutritional examination points out that *Moringa*leaves are rich in indispensable disease-preventing nutrients, which make it appropriate to be included in diets as food supplement **(**Krishnaiah *et al*., 2009). In ethnomedicine, *Moringa oleifera* leaves are mostly used by local traditional healers in treatment of different ailments especially in the

management of neurological disorders. Also, the leaves are recorded to possess antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive and antioxidant properties (Bukar *et. al.,* 2010). Previous studies have indicated the antioxidant and neuroprotective effects of *moringaoleifera*. González-Burgos *et al*., (2021), reported a preliminary evidence of the antioxidant-mediated neuroprotective effects of the methanol extract of *Moringa oleifera* leaf powder. In his studies, *Moringa oleifera* leaf powder exerted a protective effect against hydrogen peroxide in neuronal cells by reducing ROS overproduction and lipid peroxidation levels, increasing GSH content and antioxidant enzymes activity and avoiding mitochondrial dysfunction. However, regardless of its wide use traditionally and its therapeutic value, the pharmacological potential of *Moringao leifera* leaves are still largely unexplored predominantly in conditions of its role as neuroprotective agent. Therefore, considering the global neurotoxic activities of alcohol, this study aimed to explore the effects of the ethanol leaf extract of *Moringa Oleifera* (ELEMO) on the histology of the prefrontal cortex after alcohol-induced neurotoxicity.

MATERIALS AND METHODS

Plant Materials: Fresh leaves of *Moringa oleifera* were obtained from a farmland within Enugu metropolis of Enugu State. The leaves were authenticated at the Faculty of Agricultural Science, Enugu State University of Science and Technology, Enugu.

Processing of Plant Materials: The fresh leaves of *Moringa oleifera* were washed properly and left to air-dry under shade for two weeks. Afterwards, the dried leaves were pulverized to fine powder and properly sieved. The fine powder was put into an air tight container and 2.7 liters of analytical ethanol was added and stirred for 2hours and then allowed to stand for 48hours. 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.

Experimental animals: 20 healthy adult male wistar rats with an average weight of 150g-200g were procured from the animal house facility of the University of Nigeria, Enugu campus. However, 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 kept in well-ventilated breeding rooms and housed in netted iron cages. They were provided easy access to food (standard poultry mesh) and water, and were also allowed to acclimatize for 2 weeks. The animals were maintained under standard laboratory conditions and handling was done following the guidelines of the college committee for purpose of control and supervision of experiments on animals.

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 design: The rats were randomly divided into 5 groups (A-E) of 6 rats each, placed in separate cages within the Animal facility. All administrations were done orally every morning and the experiment lasted 14 days. Plant extracts were administered with the use on oral gavages and with normal saline as the vehicle. Alcohol solution was administered using a 2ml syringe with an oral cannula at the tip. Group A was the control group and was given feed and water *ad libitum* till the end of the experiment. Group B served as the untreated positive control group and was administered 2ml of 52.5% v/v aqueous alcohol solution daily for 14 days. This dosage was adopted from Olawale *et al.,* (2018). Group C and D received simultaneous administrations of 52.5% v/v aqueous alcohol solution and then 100mg/kg and 200mg/kg of the ethanol leaf extract of *Moringa oleifera* (ELEMO), respectively for 14 days. The dosage of ELEMO used for this study was adopted from Abijo *et al.,* (2019). Group E received simultaneous administrations of 52.5% v/v aqueous alcohol solution and then 100mg/kg of Vitamin E daily for 14 days.

Histological Study: The animals were sacrificed 24 hours after their last administration under ketamine (100mg/ml) as anesthesia. The whole brain tissue was carefully harvested, washed in normal saline and fixed in labeled containers for 72 hours prior to isolation of the prefrontal cortex for 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.

RESULTS

Histological Analysis

Group A: Photomicrograph of a section of the prefrontal cortex of the control animal group fed with only food and water showing the normal histoarchitecture of the prefrontal cortex. Neuronal cells cyto-architecture appears normal. H&E.X100
Group B: Photomicrograph of a section of the prefrontal cortex of the untreated animal group administered only 2ml of 52.5% v/v aqueous alcohol solution daily, showing granulations with focal area of mild glial cell infiltrations. H&E.X100
Group C: Photomicrograph of a section of the prefrontal cortex of the animal group that received simultaneous administration of 52.5% v/v aqueous alcohol solution and then 100mg/kg of the ethanol leaf extract of *Moringa oleifera* (ELEMO) showing neuronal cells and few large pyramidal cells (p). Cyto-architecture appears normal. H&E.X100
Group D: Photomicrograph of a section of the prefrontal cortex of the animal group that received simultaneous administration of 52.5% v/v aqueous alcohol solution and then 200mg/kg of the ethanol leaf extract of *Moringa oleifera* (ELEMO) showing neuronal cells and few large pyramidal cells (p). Cyto-architecture shows mild tissue granulation and mild cellular vacuolations. H&E.X100
Group E: Photomicrograph of a section of the prefrontal cortex of the animal group that received simultaneous administration of 52.5% v/v aqueous alcohol solution and then 100mg/kg of Vitamin E dailyshowing several neuronal cells. Cyto-architecture appears normal. H&E.X100

DISCUSSION

It has been noted that the prefrontal cortex undergoes functional and structural changes due to chronic alcohol consumption (Abernathy *et al*., 2010; Brodmann, 1909). Previous findings like in those of Miller and Cohen, (2000) attribute prefrontal cortex as being responsible for decision making, behavior and emotional control. Administration of 52.5% v/v aqueous ethanol solution for 14 days led to histopathological changes such as tissue granulations and focal areas of mild glial cell infiltrations within the prefrontal cortex of the rat brain. These findings are also similar the findings of Olawale *et al.,* (2018), who reported that oral administration of 2mls of 52.5% and 16.5% v/v aqueous ethanol solution respectively for 21 days led to cortical necrosis and uneven neuronal loss with varying range of vacuolations in the prefrontal cortices of the experimental animals. He associated the damages to the cerebral cortex with oxidative stress due to the acute oral alcohol intake. Simultaneous administration of aqueous ethanol solution and then 100mg/kg of Vitamin E daily showed a normal cyto-architecture of the prefrontal cortex. This indicated strong therapeutic and antioxidant effects of vitamin E. This finding is similar to previous studies by Stamm *et al.,* (2004) and Shirpoor *et al.*, (2009) who both reported that Vitamin E had a strong protective potential against alcohol-induced brain damage. Treatment with simultaneous administration of the ethanol leaf extract of *Moringa oleifera* displayed dose-dependent neuroprotective

potentials. 100mg/kg of the ethanol leaf extract of *Moringa oleifera* displayed a strong therapeutic activity as the histology of the treated animal group showed normal histo-architecture, just like that of the control group when compared to the untreated animal group. This is in accordance with previous studies by Ekong *et al.*, (2017) and Alqahtani and Albasher, (2021) who both reported that 250 mg/kg and 300 mg/kg respectively of the methanol leave extract of *Moringa oleifera* attenuated Lead (Pb) and aluminum-induced cerebral damage in rats by reducing oxidative stress, inflammation and apoptosis and by improving neurohistopathology. However, treatment with simultaneous administration of 200mg/kg of the ethanol leaf extract of *Moringa oleifera* displayed mild histopathological alterations within the prefrontal cortex such as mild tissue granulation and mild cellular vacuolations similar to the untreated animal group. This suggests that higher doses may be toxic and may lead to tissue damage. This finding is in accordance with previous studies by Abijo *et al*., (2019), who reported that high consumption of 200mg/kg of aqueous extract of *Moringa oleifera* for 6 weeks caused some slight distortions in the histoarchitecture of the frontal cortex of developing wistar rats. It has been reported that notwithstanding the plant being referred to as 'nontoxic', this does not appear to all cases (Awodele *et al*., 2012). Although, Stohs and Hartman, (2015) indicated that no adverse effects of *Moringa oleifera* were reported in association with human studies, consumption of this plant should be with caution, especially at higher doses.

CONCLUSION

This study explored the effects of the ethanol leaf extract of *Moringa oleifera* on the histology of the prefrontal cortex following alcohol-induced neurotoxicity. The findings of this study indicate that the ethanol leaf extract of *Moringa oleifera* displayed dose-dependent neuroprotective potentials. Low doses had strong therapeutic activity and protected the prefrontal cortex from histopathological alterations while high doses caused toxicity and lead to mild tissue damage. Thus, the ethanol leaf extract of *Moringa oleifera* can be used in the management of alcohol-induced neurological disorders. However, caution should still be applied while consuming this plant, especially in high quantity.

Consent: 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/ 2022/038

Competing Interests: Authors have declared that there are no competing interests.

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