International Journal of Healthcare Sciences ISSN 2348-5728 (Online)
Vol. 12, Issue 2, pp: (57-64), Month: October 2024 - March 2025, Available at: www.researchpublish.com

TANNIC ACID EXHIBITS NEUROPROTECTIVE ABILITIES IN THE MICROSTRUCTURES OF THE PREFRONTAL CORTEX OF ADULT WISTAR RATS FOLLOWING ETHANOL-INDUCED TOXICITY

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DOI: https://doi.org/10.5281/zenodo.14509552

Published Date: 17-December-2024

Abstract: **Background: High alcohol intake is a known cause of brain damage. Tannic acid (TA) poses as a potential treatment measure. Aim: To investigate the histomorphological changes in the microstructures of the prefrontal cortex of adult Wistar rats during treatment with tannic acid following ethanol-induced neurotoxicity. Methodology: Thirty six (36) adult male Wistar rats (160g-240g) were assigned into six (6) groups (A to F) of 6 rats each. Group "A" (untreated negative control) received daily doses of distilled water at 6ml/kg/bwt while Group "B" (alcohol control group) received daily doses of 6g/kg/bwt of 40% ethanol only. Group "C, D and E" received daily doses of 6g/kg/bwt of 40% ethanol co-administrated with 200mg/kg/bwt, 100mg/kg/bwt and 50mg/kg/bwt of TA respectively. Group "F" (positive control group) received daily doses of 6g/kg/bwt of 40% ethanol co-administrated with 335mg/kg/bwt of Vitamin E. All treatments were oral and lasted 14 days. The animals were sacrificed under intraperitoneal ketamin/thiopental sodium as anaesthesia after 24 hours of fasting. Brain tissues were quickly but carefully removed and fixed in 10% formal saline. The prefrontal cortex was grossed and processed according to standard protocols, sectioned at 5µm thick for standard histological studies. Result: Histomorphological analysis demonstrates that high and medium doses of TA preserve neuronal integrity and mitigate the damaging effects of ethanol exposure. Treatment with vitamin E suggests that while antioxidant properties may aid in neuronal preservation, they do not fully address the inflammatory responses triggered by ethanol. Conclusion: Tannic acid exhibits comparatively better neuroprotective effects against ethanol-induced prefrontal cortex toxicity.**

Keywords: **Tannic acid, Ethanol toxicity, Prefrontal Cortex, Histomorphology, Wistar rats.**

1. INTRODUCTION

Since the existence of man till date, plants have been used as a source of medicine for different health purposes. The phyto-constituents of many plant species, including glycosides, alkaloids, saponins, steroids, flavonoids, tannins, and terpenoids (e.g., monoterpenes, diterpenes, and sesquiterpenes), have been shown to exert pharmacological effects [1,2].

Medicinal plant extracts and their constituents have been shown to have virucidal, bactericidal, fungicidal, anti-inflammatory, analgesic, sedative, and local anesthetic properties [3,1,2]. Tannic acid (TA) is a naturally occurring large polyphenol, found in several herbaceous and woody plants, wines, and a broad selection of teas [4]. TA is found to exert various biological effects such as being antioxidant [5], neuroprotective [6], antiinflammatory, anticarcinogenic, antiapoptotic as well as antitumoral and antiviral effects [7,8,9].

Alcohol is the most widely used substance of abuse. It results in 30% deaths per year in males aged 15-29 years [10,11]. Binge ethanol administration has been shown to produce neurodegeneration by inducing necrosis in corticolimbic regions in rats [11,12]. Recent work has demonstrated a decrease in functional heterogeneity in right prefrontal cortex and other parts of the brain following ethanol administration [13]. Thus, this research aimed at utilizing the routine H&E as well as Cresyl-fast-violet staining protocol to investigate the histomorphological changes in the microstructures of the prefrontal cortex of adult wistar rats treated with tannic acid following ethanol-induced toxicity using.

2. MATERIALS AND METHODS

Experimental Animals

This study was carried out in the Animal facility of the Enugu State University of Science and Technology College of Medicine, Parklane, Enugu. Thirty (36) adult male wistar rats weighing between 160g-240g were procured and assigned into six (6) groups (A to F) of 6 rats each. The animals were kept in well ventilated breeding rooms and housed in netted iron cages. There were allowed to acclimatize for 2 weeks while provided easy access to food and water *ad libitum*. The experimental protocols and techniques for this study were carried out in accordance with the standard principles of international animal use and care. Ethical approval was gotten from the university's ethical clearance committee with the ethical right permission number: ESUCOM/FBMS/ETR/2024/003.

Experimental Design

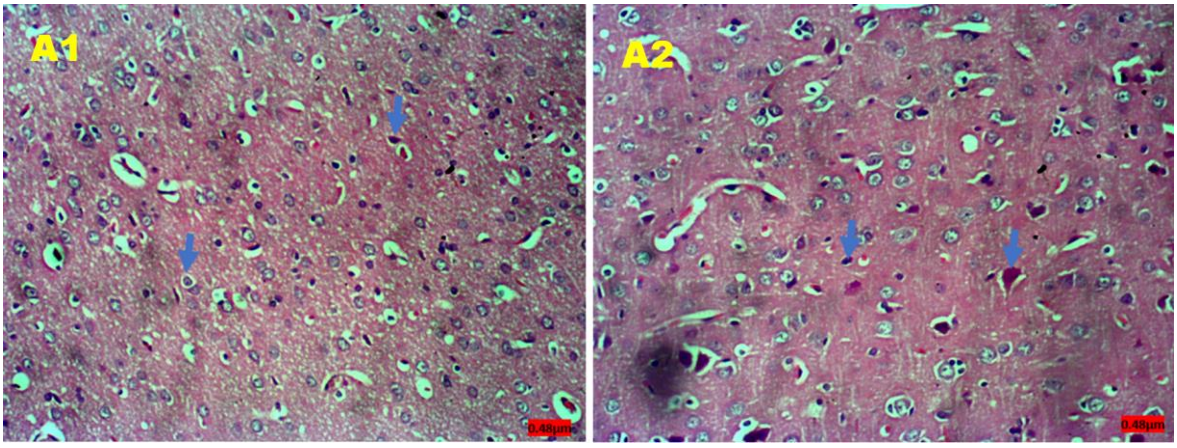
Each animal group was placed in separate cage within the Animal facility. All treatments were carried out orally and were performed daily for 14 days. Group "A" rats represented the untreated (negative) control and received daily doses of distilled water at 6ml/kg/bwt while Group "B" rats received daily doses of 6g/kg/bwt of 40% ethanol only; representing the alcohol control group [14,15]. Group "C, D and E" rats received daily doses of 6g/kg/bwt of 40% ethanol co-administrated with 200mg/kg/bwt, 100mg/kg/bwt and 50mg/kg/bwt of Tannic acid respectively [14,15]. Accordingly, group "F" rats also received daily doses of 6g/kg/bwt of 40% ethanol co-administrated with 335mg/kg/bwt of Vitamin-E as a standard drug; representing positive control group [16].

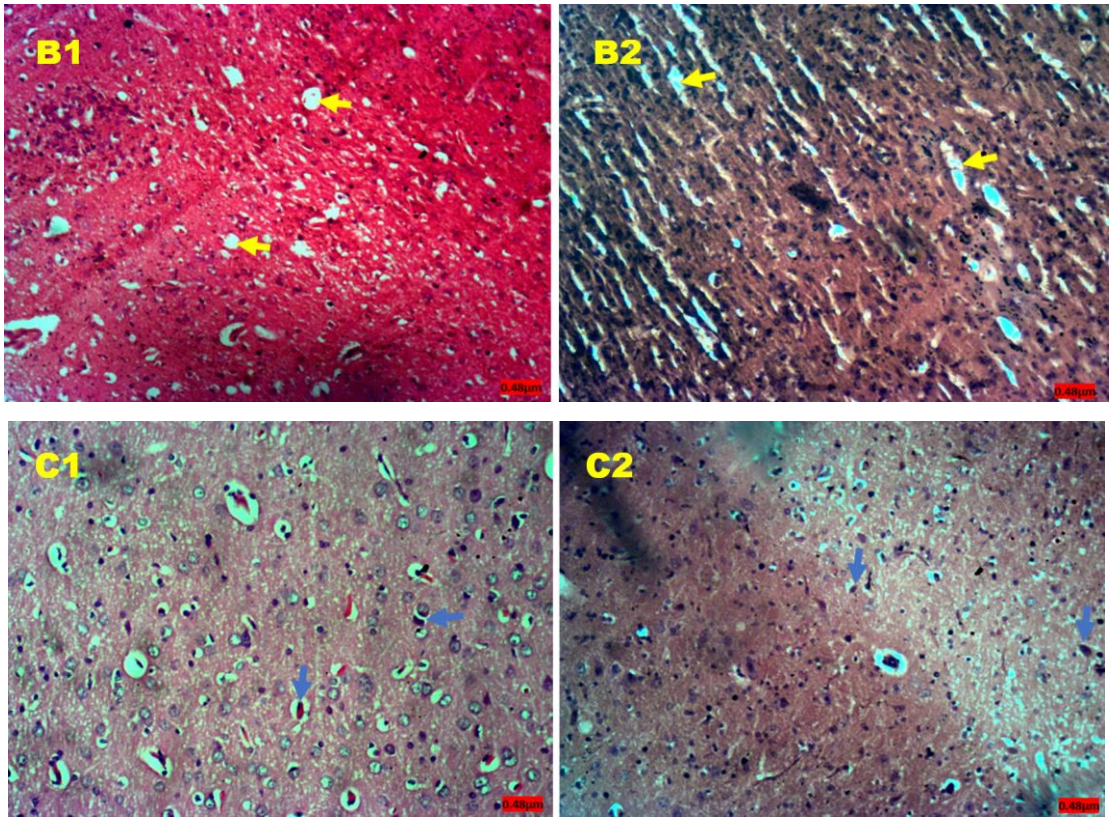
Histological Study

The animals were sacrificed using 60/30mg/kg/bwt of intraperitoneal ketamin/thiopental sodium as anaesthesia after 24 hours of fasting [17]. The skulls were dissected under anaesthasia and brain tissues were quickly but carefully removed and fixed in 10% formal saline. After 48 hours of fixation in labeled containers, the prefrontal cortex was grossed and further re-fixed in 10% formal saline for histological studies. The tissues were processed according to standard protocols with sectioning systematically sampled at 5µm thick for standard staining protocols using H&E as well as Cresyl fast violet methods for histological studies [18]. Photomicrography was employed using Amscope digital camera (version 3.7) at x300 magnification and a scale of 0.48µm.

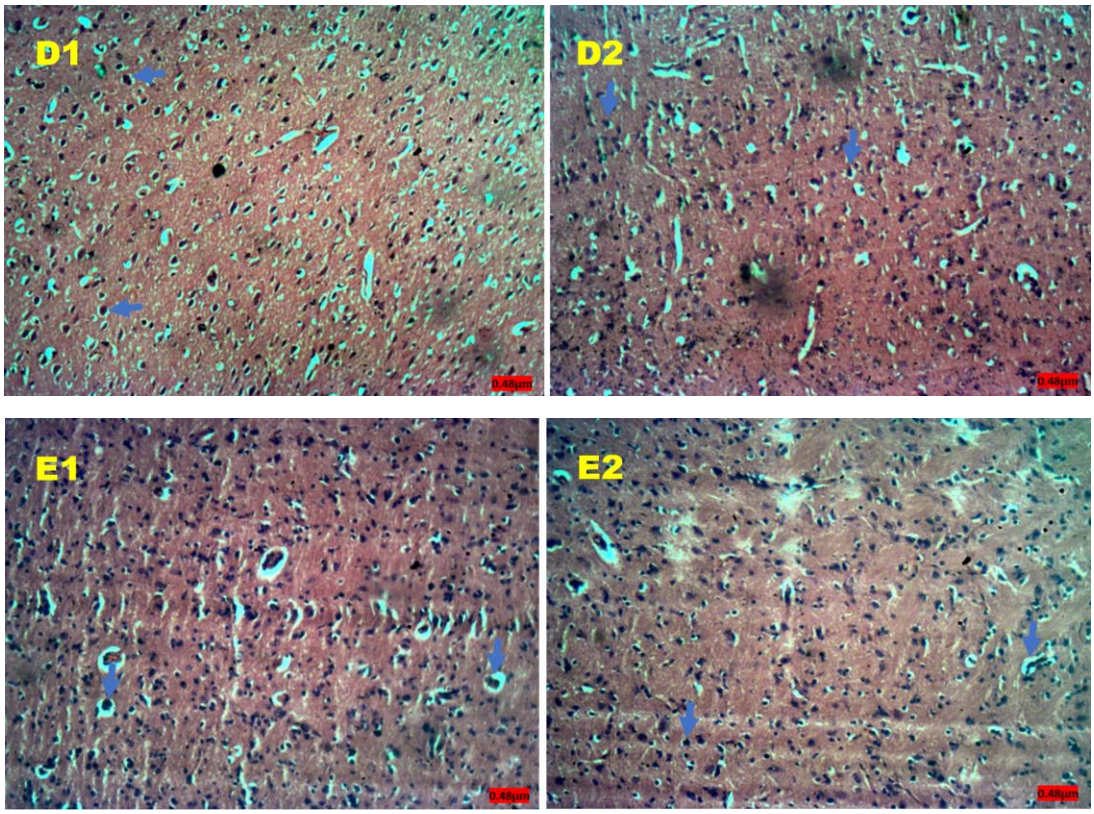
3. RESULTS

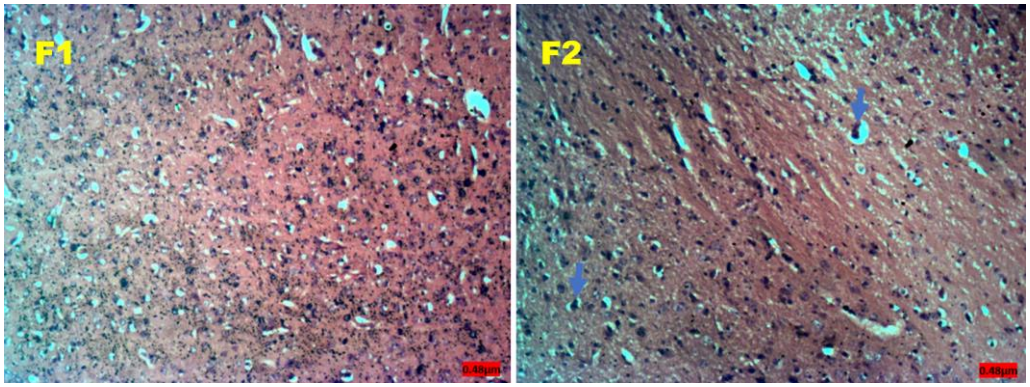
H&E Findings





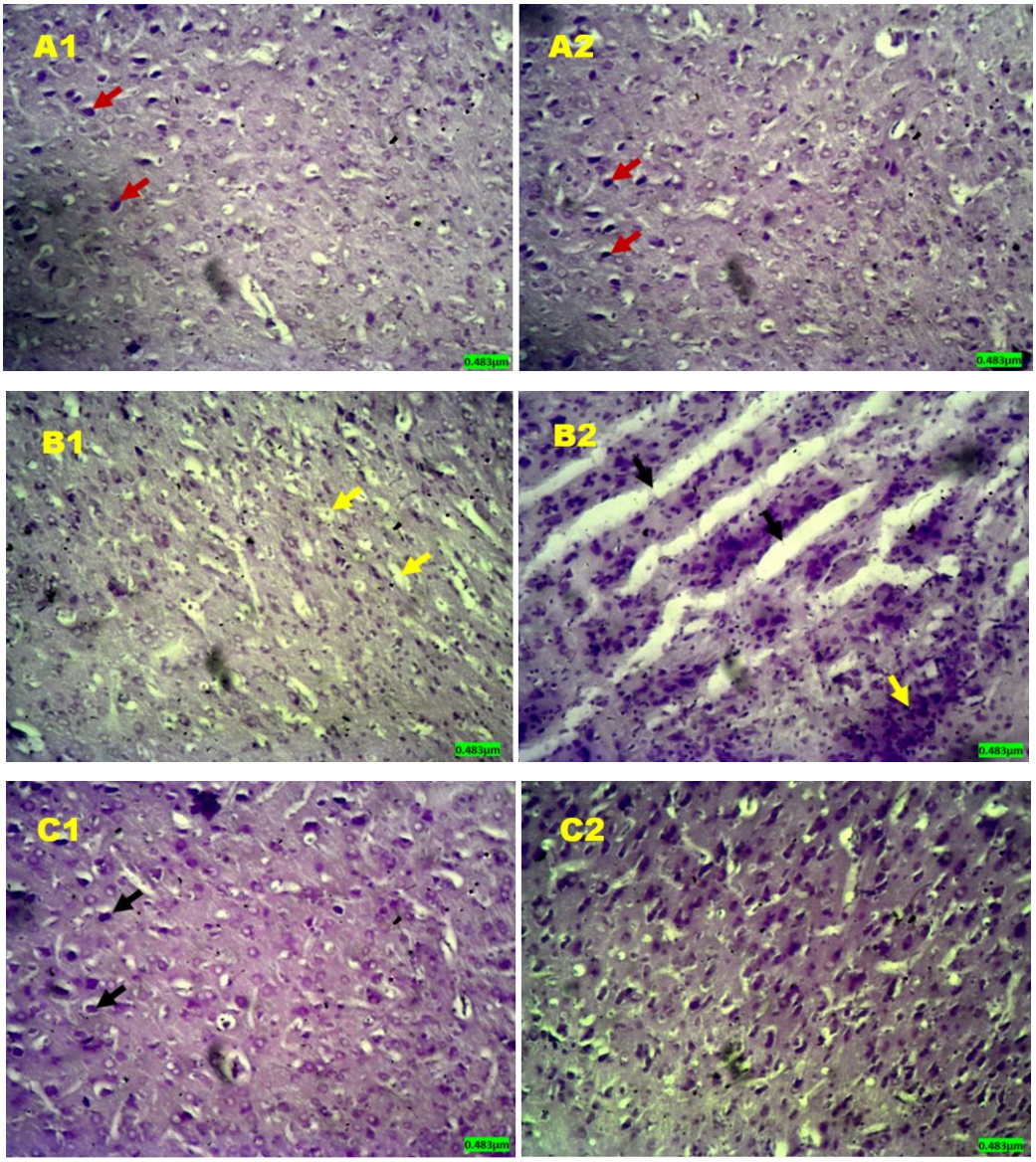
Photomicrographs of the prefrontal cortex microstructure of the experimental animals. **Group A:** Control animal group showing interspersed neuronal cells (arrow). Cytoarchitecture appears normal. **Group B:** Untreated animal group showing focal areas of cortical laminar necrosis (B1 arrow) and tissue scarring due to cerebrovascular reactivity (B2 arrow). **Group C:** Animal group treated with 200mg/kg/bwt of Tannic acid showing interspersed neuronal cells (arrow). Cytoarchitecture appears normal. **H&E:x300. SCALE: 0.48µm**



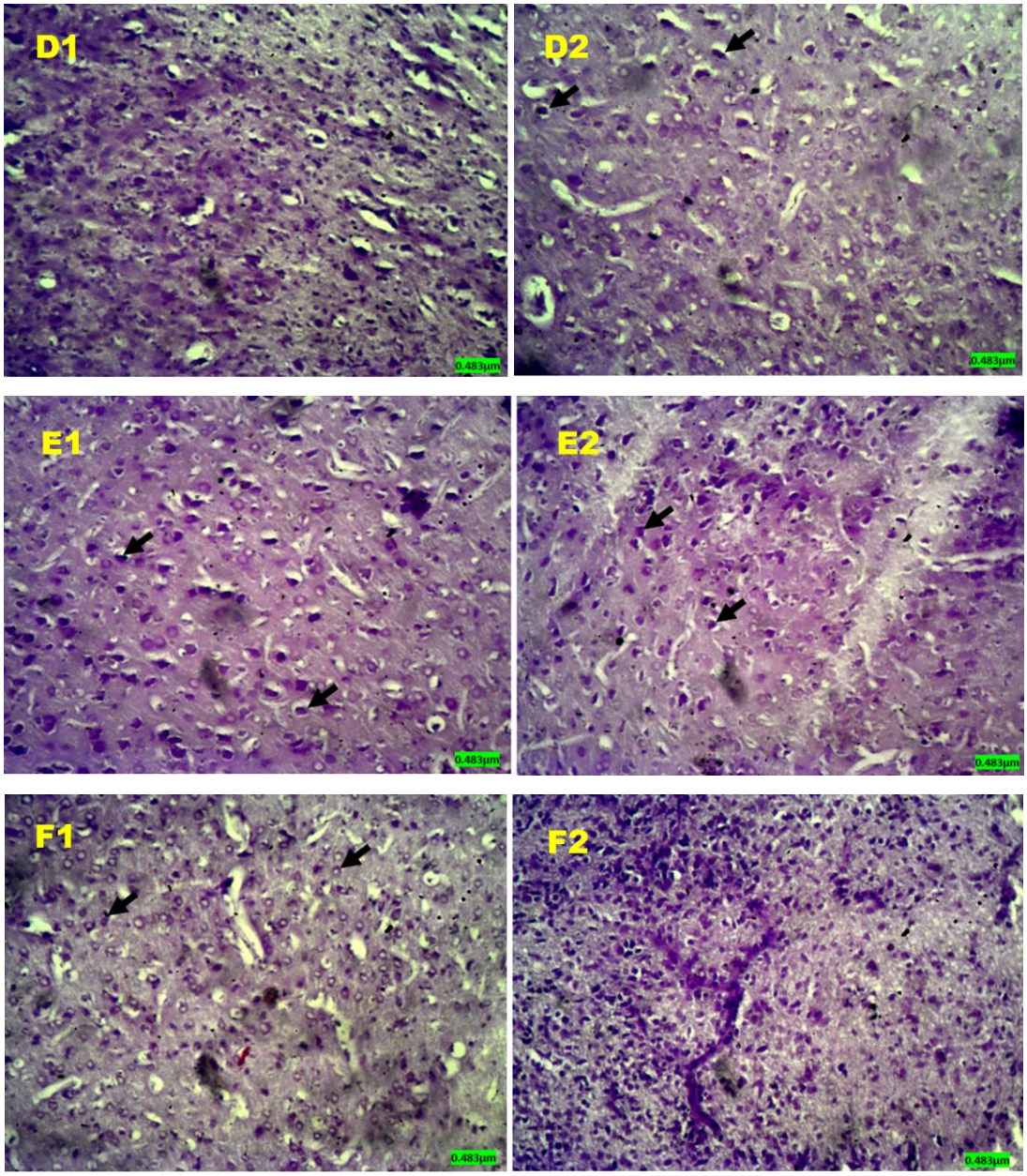


Photomicrographs of the prefrontal cortex microstructure of the experimental animals. **Group D:** Animal group treated with 100mg/kg/bwt of Tannic acid showing interspersed neuronal cells (arrow). Cytoarchitecture appears normal. **Group E:** Animal group treated with 50mg/kg/bwt of Tannic acid showing interspersed neuronal cells (arrow). Cytoarchitecture appears normal. **Group F: A**nimal group treated with 335mg/kg/bwt of Vitamin-E as a standard drug; showing prominent glial cell activation (F1) and interspersed neuronal cells in F2 (arrow). Cytoarchitecture appears normal. **H&E:x300. SCALE: 0.48µm**

Cresyl fast violet Findings



Photomicrographs of the prefrontal cortex microstructure of the experimental animals. **Group A:** Control animal group showing normal distribution of neurons (arrow). **Group B:** Untreated animal group showing mild depletion of neurons (B1 arrow) and severe neuronal depletion (B2) and glial cell activation (yellow arrow). **Group C:** Animal group treated with 200mg/kg/bwt of Tannic acid showing normal distribution of neurons (C1) and mild glial cell activation (C2). **CFV:x300. SCALE: 0.48µm**



Photomicrographs of the prefrontal cortex microstructure of the experimental animals. **Group D:** Animal group treated with 100mg/kg/bwt of Tannic acid showing mild glial cell activation (D1) and normal distribution of neurons (D2). **Group E:** Animal group treated with 50mg/kg/bwt of Tannic acid showing normal distribution of neurons (arrow). **Group F: A**nimal group treated with 335mg/kg/bwt of Vitamin-E as a standard drug; showing normal distribution of neurons (F1) and severe glial cell activation (F2). **CFV:x300. SCALE: 0.48µm**

4. DISCUSSION

At 29% of the total cerebral cortex, the prefrontal cortex (PFC) is the biggest cortical area in the human brain situated in the frontal lobe, in front of the premotor and primary motor cortices [19]. PFC plays a major part in determining behavior and personality. In addition, it performs a wide range of other tasks, such as working memory, temporal perception, speech, attention maintenance, complicated movement planning, emotion regulation, and moral judgment [20,19]. According to

Abernathy *et al*., [21] and Tu et al., [22], the PFC's working memory is responsible for storing short-term memory data, temporal perception, and foresight, prospective planning, determining whether a stimulus is behavioral, preparing a planned and logical response to a sensory signal, solving complex mathematical problems, and determining whether a behavior is morally acceptable. It has been demonstrated that PFC is essential for both retrieving recent memories and retaining prior knowledge [23].

Normal physiological conditions (A1 and A2) preserve neuronal integrity which is seen as interspersed neuronal cells; indicating a healthy cytoarchitecture and stands as a baseline for comparison with the other treatment groups. Our findings from the untreated groups demonstrated significant pathological changes, including focal areas of cortical laminar necrosis and tissue scarring due to cerebrovascular reactivity. These findings indicate that ethanol exposure resulted in substantial neuronal damage and altered tissue integrity, suggesting that ethanol induces neurotoxic effects on the prefrontal cortex as previously recorded by previous literatures by Du et al., [24], Tizabi et al., [25] and Udonkang et al., [26] who reported similar findings.

Treatment with 200mg/kg/bwt of TA upon ethanol-induced neurotoxicity preserved the cytoarchitecture of the prefrontal cortex (C1 and C2); suggesting that high-doses tannic acid effectively counteracts the toxic effects of ethanol, promoting neuroprotection and the maintenance of normal neuronal distribution. This ability can be attributed to the anti-oxidative potentials and other special qualities of TA as supported by previous studies. Kim et al., [27] reported that TA provides sufficient neuroprotection in animal models of stroke (transient middle cerebral artery occlusion; tMCAO) and demonstrates Zn2+-chelating and anti-oxidative capabilities in primary cortical neurons. Also, Karagac and Ceylan, [28] demonstrated that dietary intakes of TA significantly mitigated monosodium glutamate-induced dysregulation in cortical tissue by regulating redox balance, cellular homeostasis, and controlled cell death. Treatment with 100mg/kg/bwt and 50mg/kg/bwt demonstrated partial protective capabilities. While neuronal structures remain intact, the mild glial activation may suggest a reactive response to ethanol toxicity that the tannic acid is addressing, albeit not as robustly as the higher doses. In comparison with treatments with Vitamin E, a normal distribution of neurons was noticed alongside severe glial cell activation. This discrepancy implies that while vitamin E may help in preserving neuronal structures, it does not prevent glial activation, potentially indicating an inflammatory or reactive process still at play in the presence of ethanol. This however supports the neuroprotective abilities of vitamin E as reported by Khanna et al., [29] and La Torre et al., [30] who demonstrated the 'Role of vitamin E in neuroprotection. Marinelli et al., [31] also reported the effective neuroprotective potentials of Vitamin E in epilepsy cases.

The histomorphological findings substantiate the hypothesis that tannic acid can mitigate ethanol-induced damage in the prefrontal cortex of adult Wistar rats. The preservation of normal cytoarchitecture in groups treated with 200mg/kg/bwt and 100mg/kg/bwt of tannic acid emphasizes its protective role against neurotoxicity. The presence of glial activation, especially following treatment with 100mg/kg/bwt, hints at an ongoing protective or reparative response to neuronal stress, possibly serving as a compensatory mechanism. In contrast, the ethanol-only group underscores the damaging effects of ethanol, characterized by necrosis and scarring. The protective effect of tannic acid, particularly at higher doses, highlights its potential as a therapeutic agent in managing ethanol-induced neurotoxicity. This in tandem to a related study which observed that tannic acid (TA) are more effective if taken parallel to food contaminated by Cadmium and their effectiveness is higher if their intake is long-term [32]. Another study reported the neuroprotective abilities of tannic acid against traumatic brain injury via the PGC-1α/Nrf-2/HO-1 signaling pathway [33]. Moreover, the findings in the treatment with vitamin E suggest that while antioxidant properties may aid in neuronal preservation, they do not fully address the inflammatory responses triggered by ethanol. These facts points to the complexity of neuroprotection, where multiple pathways may be involved in neuronal survival and glial activation. A study reported the protective abilities of tannic acid from glial cell activation and protected rat brain from poisonous effect of Lead-acetate exposure in experimental rats [34].

5. CONCLUSION

Tannic acid exhibits neuroprotective effects against ethanol-induced toxicity in the prefrontal cortex of adult male Wistar rats. The histomorphological analyses demonstrated that high and medium doses of tannic acid preserve neuronal integrity and mitigate the damaging effects of ethanol exposure.

Conflict of interest

This study is not associated with any conflict of interest.

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