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NEUROPROTECTIVE EFFECTS OF TANNIC ACIDS ON ASTROCYTES AND NEURONAL CELL POPULATION IN NEURAL CREST CELLS OF THE PREFRONTAL CORTEX FOLLOWING ETHANOL-INDUCED PREFRONTAL CORTEX TOXICITY; AN IMMUNOHISTOCHEMICAL STUDY

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Abstract: **Background: Alcoholism has been associated with brain damage especially in distinct brain regions such as the prefrontal cortex. Aim: To explore the astrocytes and neuronal cell population of the Prefrontal Cortex treated with tannic acid (TA) following ethanol-induced prefrontal cortex toxicity using standard GFAP and NeuN immunihistochemisty protocols. 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 GFAP and NueN immunohistochemistry studies. Result: TA, particularly in higher doses, offers comparable, if not superior neuroprotective effects than Vitamin-E. Across different dosages, TA mitigated ethanol-induced neuronal loss and promoted neuronal survival and differentiation in the prefrontal cortex. Conclusion: Tannic acid posed as a potential therapeutic agent in treating ethanol-induced neurotoxicity, with efficacy demonstrated even at lower doses.**

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

1. INTRODUCTION

Throughout history, heavy alcohol consumption has been associated with brain damage; an indication that the brain is a key target for the action of alcohol [1]. Patients and animal models have been used to demonstrate the pathological changes in brain anatomy and neuronal functions caused by ethanol especially in several distinct brain regions, including the frontal

cortex, hippocampus, and cerebellum [2,3,4]. Tannic acids have demonstrated anti-inflammatory and anti-oxidant Actions [5,6], is seen to be potent against cerebral ischemic injury [7] and possess other brain-related therapeutic functions in experimental animal trials [8]. In comparison with other neuroprotective agents such as Vitamin E, this study aims to determine the mean count of astrocytes and neuronal cell population in the microstructure of neural crest cells of Prefrontal Cortex of adult wistar rats treated with tannic acid, following ethanol-induced prefrontal cortex toxicity using standard immunihistochemisty protocols.

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 cages 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 [9,10](Sharma *et al.*, 2012 and Azam Ramezani *et al.*, 2012). 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 [9,10]. 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 [11].

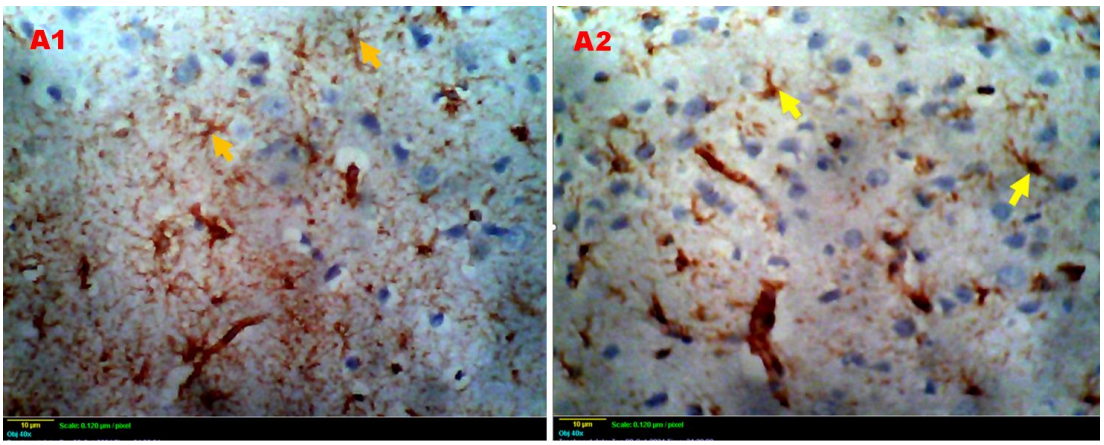
Immunohistochemical Study

The animals were sacrificed using 60/30mg/kg/bwt of intraperitoneal ketamin/thiopental sodium as anaesthesia after 24 hours of fasting [12]. 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 routine histological staining as well as GFAP and NueN immunohistochemistry [13,14]. Photomicrography was employed using Amscope digital camera (version 3.7).

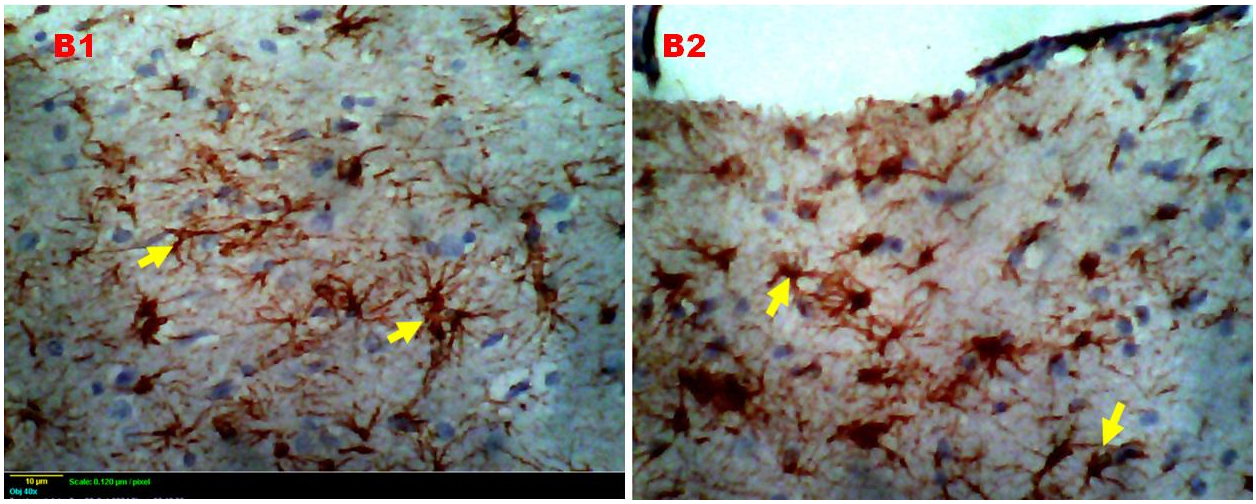
3. RESULTS

Immunohistochemical Analysis

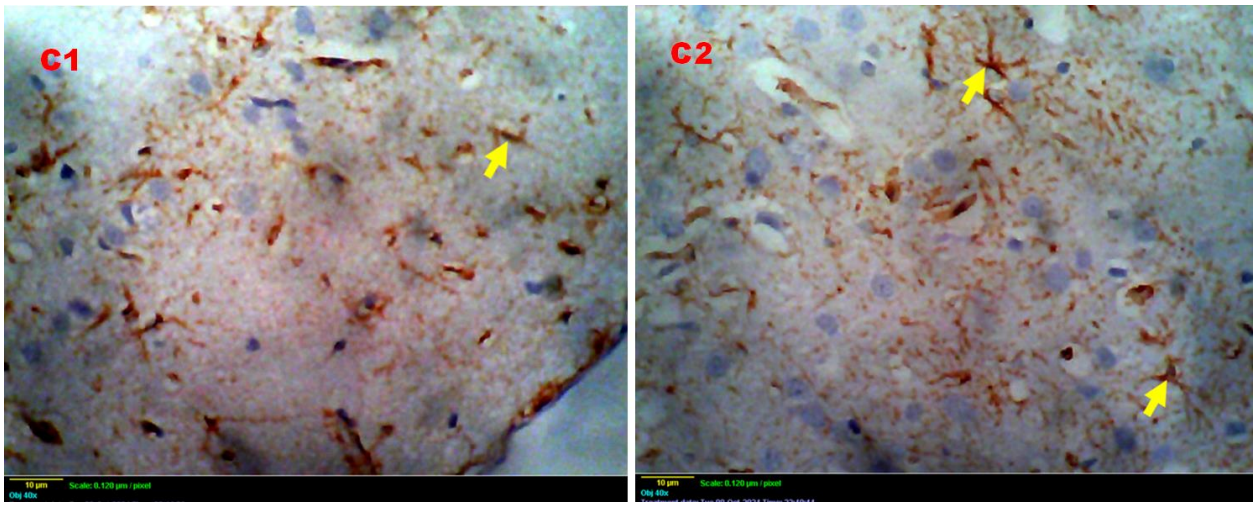
GFAP Results: Mag: x400



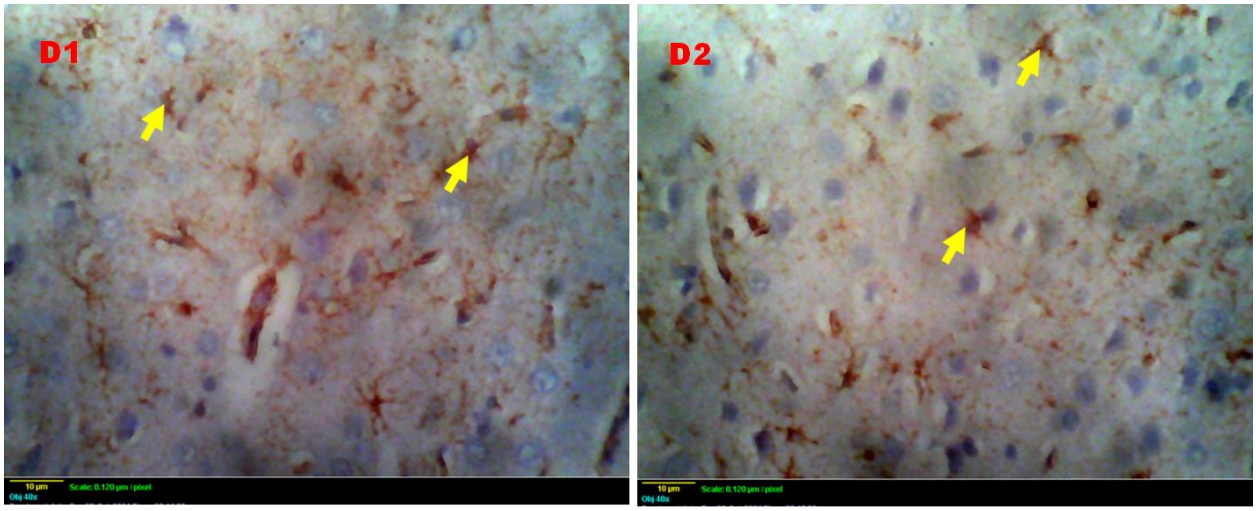
Group A: Photomicrograph of the prefrontal cortex of the control animal group showing normal distribution of astrocytes (arrow).



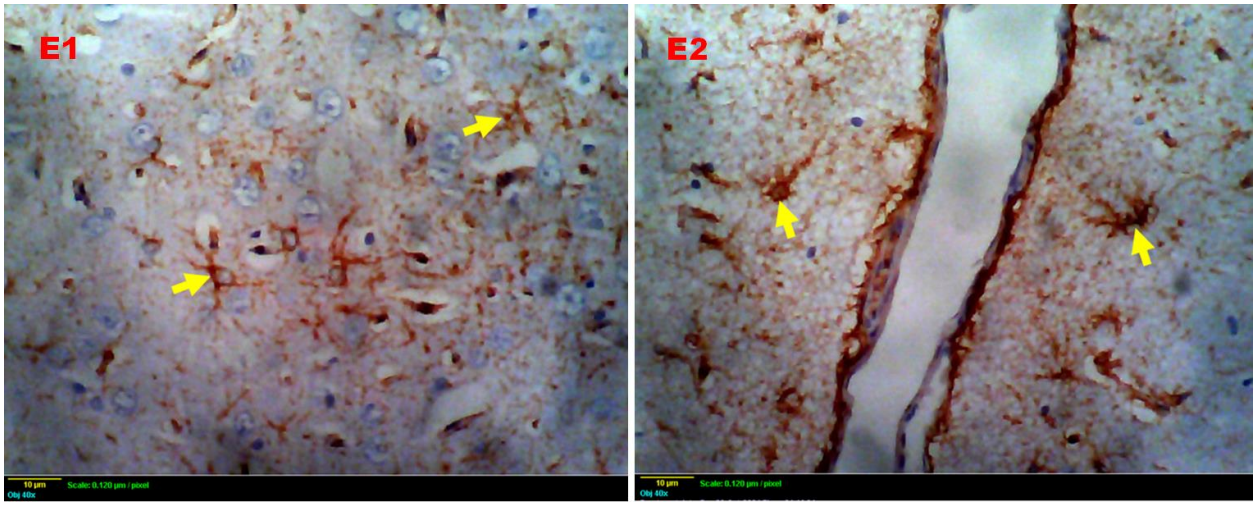
Group B: Photomicrograph of the prefrontal cortex of the untreated animal group showing moderate (B1) and prominent (B2) expression of astrocytes (arrow).



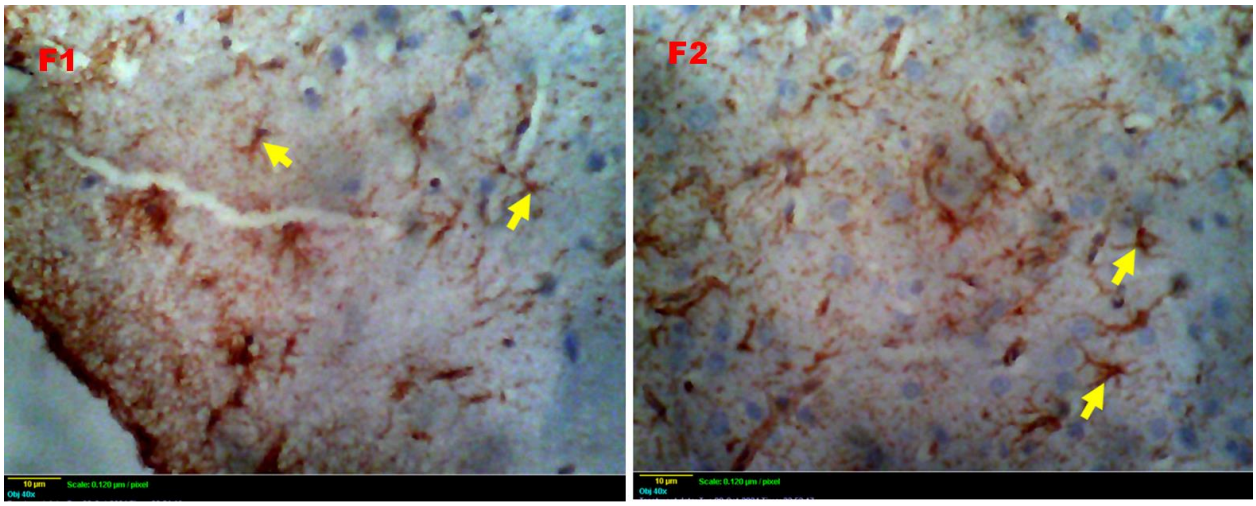
Group C: Photomicrograph of the prefrontal cortex of the animal group treated with 200mg/kg/bwt of Tannic acid showing mild expression of astrocytes (arrow).



Group D: Photomicrograph of the prefrontal cortex of the animal group treated with 100mg/kg/bwt of Tannic acid showing mild expression of astrocytes (arrow).

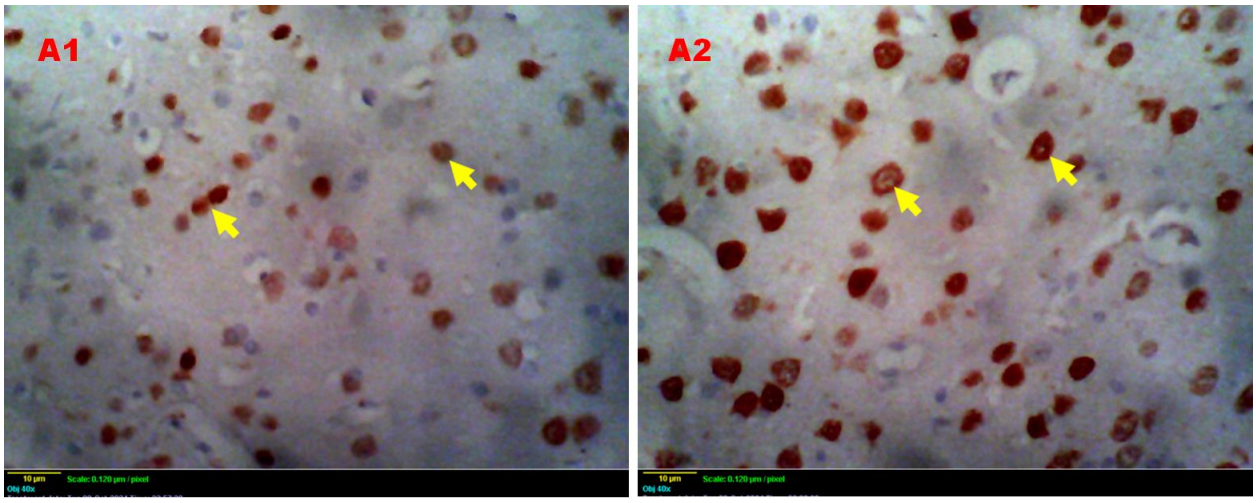


Group E: Photomicrograph of the prefrontal cortex of the animal group treated with 50mg/kg/bwt of Tannic acid showing moderate expression of astrocytes (arrow).

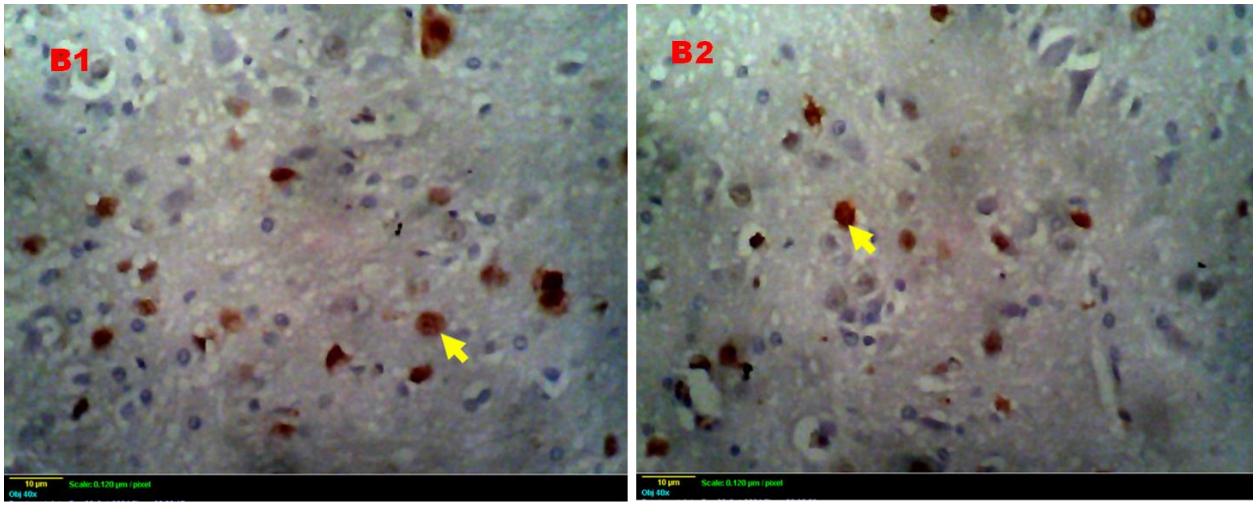


Group F: Photomicrograph of the prefrontal cortex of the animal group treated with 335mg/kg/bwt of Vitamin-E as a standard drug; showing moderate expression of astrocytes (arrow).

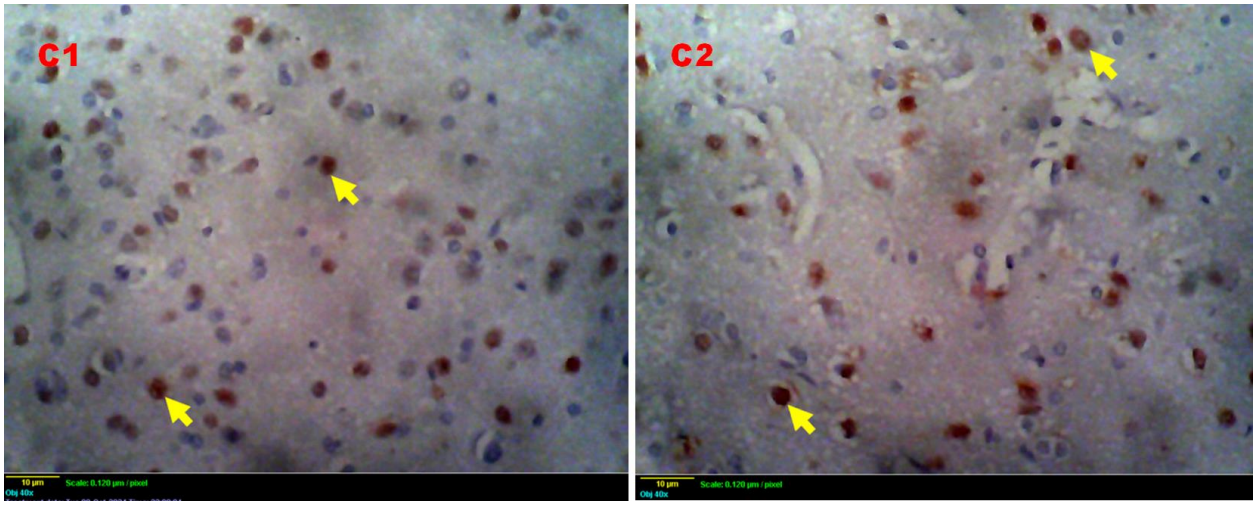
NeuN Results: Mag: x400



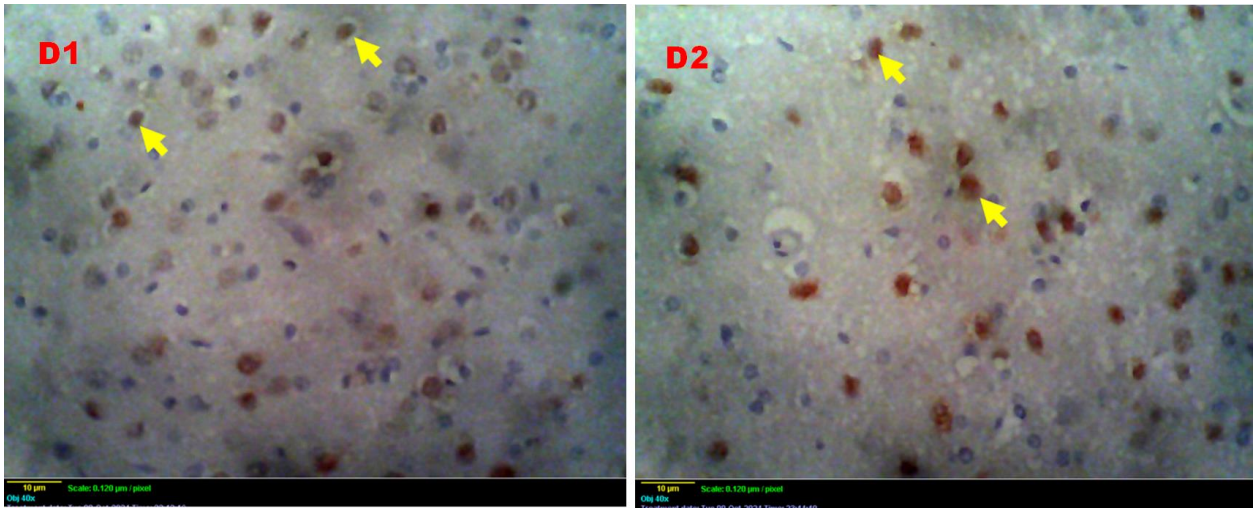
Group A: Photomicrograph of the prefrontal cortex of the control animal group showing average (A1) and prominent (A2) neuronal differentiation to NeuN antibody (arrow).



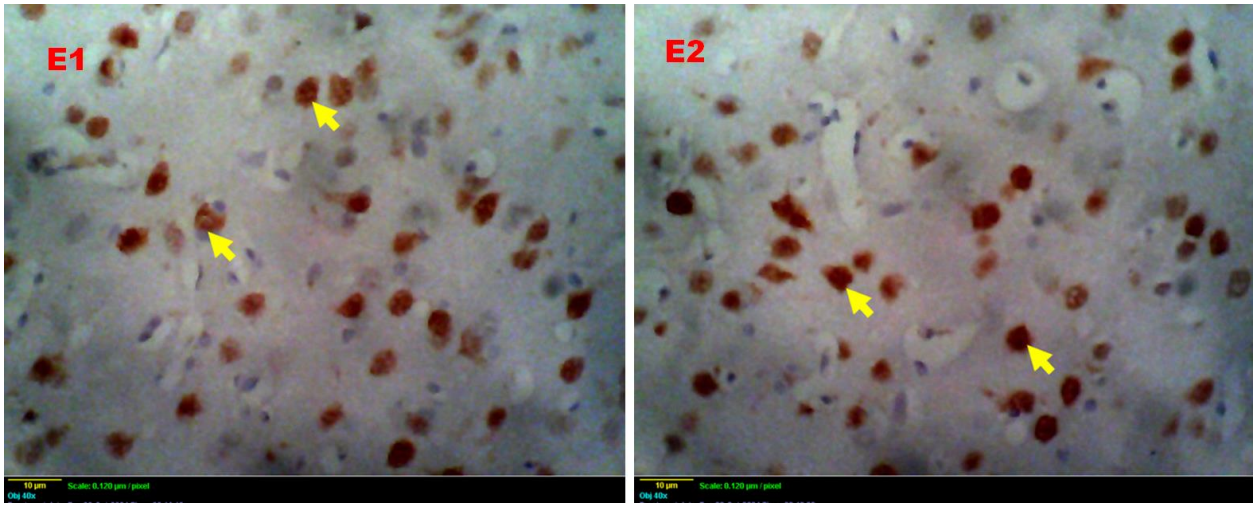
Group B: Photomicrograph of the prefrontal cortex of the untreated animal group showing mild neuronal differentiation to NeuN antibody (arrow).



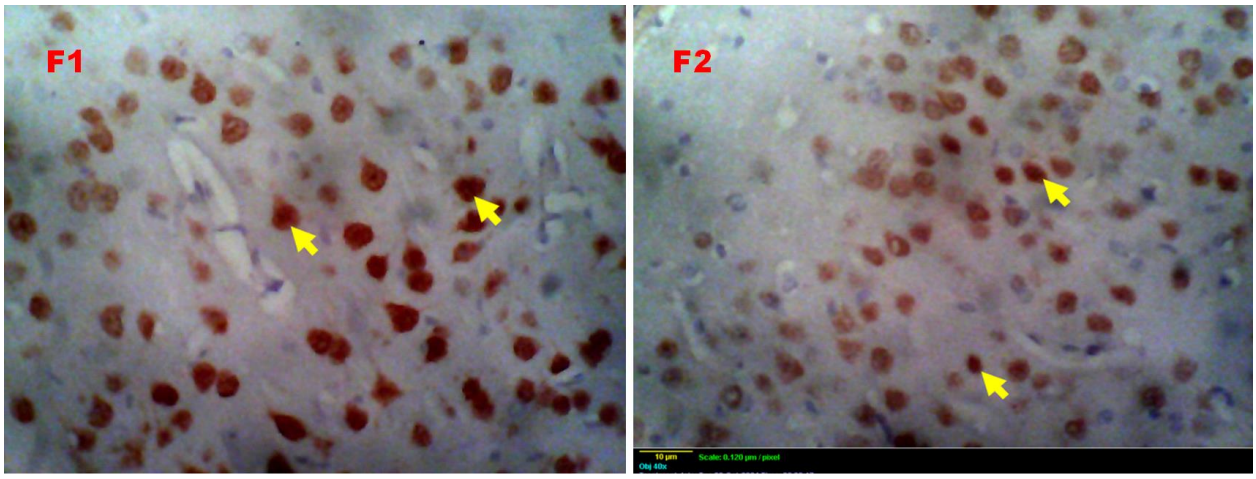
Group C: Photomicrograph of the prefrontal cortex of the animal group treated with 200mg/kg/bwt of Tannic acid showing average neuronal differentiation to NeuN antibody (arrow).



Group D: Photomicrograph of the prefrontal cortex of the animal group treated with 100mg/kg/bwt of Tannic acid showing average neuronal differentiation to NeuN antibody (arrow).



Group E: Photomicrograph of the prefrontal cortex of the animal group treated with 50mg/kg/bwt of Tannic acid showing prominent neuronal differentiation to NeuN antibody (arrow).



Group F: Photomicrograph of the prefrontal cortex of the animal group treated with 335mg/kg/bwt of Vitamin-E as a standard drug; showing great/prominent neuronal differentiation to NeuN antibody (arrow).

4. DISCUSSION

The prefrontal cortex plays a fundamental role in numerous higher-order cognitive functions [15]. It is also responsible for social cognition, emotional regulation as well as memory [16]. Thus, its functionality is essential for complex performances, innovation and social learning [17,18]. Nevertheless, it is a distinct brain region known to be affected in the advent of alcohol-related brain damage [2].

The Glial Fibrillary Acidic Protein (GFAP) immunohistochemistry protocol is a crucial marker for astrocytes, involved in neural repair, maintenance, and injury response, including glial cell activation in response to neurotoxicity [19,20]. Photomicrographs of the prefrontal cortex microstructure in the normal control group (A1 and A2) show healthy neuronal density and no neurotoxicity, serving as a baseline comparison. The ethanol exposure causes mild neuronal depletion in B1, while severe neuronal depletion in B2 is evident, indicating glial cell activation and cumulative neurodegenerative impact as demonstrated in previous studies by [21] and [22].

The group with high dose of TA shows significant protection against ethanol-induced neuronal depletion in C1 neurons, preventing extensive loss. However, mild glial cell activation suggests that tannic acid maintains neuronal structure and minimizes excessive astrocyte activation, reflecting the body's normal neuroprotective mechanisms. Medium doses of tannic acid offer partial protection against ethanol-induced neuronal damage, maintaining neuronal integrity while triggering some astrocyte response, with mild glial cell activation observed in D1 and a normal distribution of neurons in D2. These observations were in tandem with a related study which showed the number of activated astrocytes in traumatic brain injury was inhibited by TA in the PGC-1α/Nrf2/HO-1 Pathway [23]. Low doses of tannic acid, even when ingested,

may protect against ethanol-induced neuronal damage and maintain neural homeostasis without over-activating astrocytes. These doses may preserve neuronal structure through antioxidative properties. A previous report demonstrated that 50 mg/kg/day of TA induced in vitro selective antiglioma activity, not demonstrating cytotoxicity in astrocyte culture and the antiglioma effect of TA was also observed in vivo, as TA decreased tumor volume by 55%, accompanied by an increase in the area of intratumoral necrosis and infiltration of lymphocytes without causing systemic damage [24].

Vitamin E protects against ethanol-induced neuronal damage, but severe glial cell activation is observed, suggesting it preserves neurons but does not inhibit astrocyte activation, possibly due to on-going neuroinflammatory processes or repair efforts. The results across all groups indicate that tannic acid, particularly in medium to high doses, provides neuroprotection by preserving the neural architecture of the prefrontal cortex and minimizing astrocyte activation compared to ethanol-only exposure. In contrast, Vitamin E, while effective in preventing neuronal loss, does not appear to prevent excessive glial activation. This finding suggests that tannic acid may have a dual role: preventing both neuron loss and excessive astrocyte activation.

The NeuN imunohistochemical findings demonstrate that ethanol exposure (B1 & B2) severely impairs neuronal differentiation in the prefrontal cortex, consistent with the known neurotoxic effects of ethanol [25]. Ethanol-induced neurodegeneration is evident through the reduced response to NeuN staining, highlighting a diminished neuronal cell population and impaired differentiation. TA, especially at higher doses (C1, C2), shows a protective effect by preserving neuronal differentiation, as evidenced by the return to average differentiation levels. The prominent neuronal differentiation observed in the low-dose group (E1, E2) is particularly notable, suggesting that even lower doses of TA are effective in maintaining neural integrity. The Vitamin E group (F1, F2) exhibited great neuronal differentiation, underscoring the efficacy of Vitamin E as a neuroprotective agent in line with the reports of [26,27] as well as [28]. However, the data also reveal that tannic acid, particularly in higher doses, offers comparable, if not superior, neuroprotective effects in this context. This in tandem with a related study which reported that treatment with TA prevents memory deficits and reestablishes Akt and pAkt expression, protecting against neuronal death and neuroinflammation in STZ-induced SDAT in rats [29].

5. CONCLUSION

The overall findings indicate that tannic acid, across different dosages, help to mitigate ethanol-induced neuronal loss and promote neuronal survival and differentiation in the prefrontal cortex. This protective effect likely arises from tannic acid's antioxidative and neuroprotective properties, which help maintain the structural and functional integrity of neurons. Therefore, tannic acid could be a potential therapeutic agent in treating ethanol-induced neurotoxicity, with efficacy demonstrated even at lower doses. However, the specific mechanisms behind tannic acid's neuroprotective effects are not known.

Conflict of interest

This study is not associated with any conflict of interest.

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