ISSN 2349-7823

International Journal of Recent Research in Life Sciences (IJRRLS)
Vol. 11, Issue 4, pp: (34-40), Month: October - December 2024, Available at: www.paperpublications.org

EFFECTS OF TANNIC ACID TREATMENTS ON ETHANOL-INDUCED PREFRONTAL CORTEX TOXICITY: A NEUROBEHAVIOURAL INVESTIGATION

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DOI: https://doi.org/10.5281/zenodo.14552691

Published Date: 24-December-2024

Abstract: **Background: Animal models have demonstrated brain damage upon ethanol intoxication. Tannic acid (TA) demonstrates various medicinal capabilities including neuroprotective potentials. Aim: To investigate the neurobehavioral effects of TA treatments following ethanol-induced prefrontal cortex toxicity. 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. Neurobehavioural investigation was conducted on day 15 via an Open field test (OFT), an Elevated plus maze test (EPM), and a Y-maze test respectively. Following statistical analysis, using one-way ANOVA, p–value≤0.05 was considered as statistically significant. Result: No statistically significant difference was recorded in both time spent in the center and in the periphery during the OFT. No statistically significant difference was recorded in the number of open and closed arm entries in the EPM. No statistically significant difference was recorded in mean percentage alternation between the treatment and control groups in the Y-maze Test. Conclusion: TA did not mitigate or worsen the neurobehavioural effects of ethanol-induced prefrontal cortex toxicity, maintaining the insights on its neuroprotective potentials.**

Keywords: **Tannic acid, Neurobehavioral effects, Prefrontal cortex, Wistar rats.**

1. INTRODUCTION

Behavior is complex as it entails an action, reaction or attitude of an individual in response to a stimuli or impending situation. It is the outcome of multilevel brain integration and has been linked to certain parts of the brain especially the prefrontal cortex [1,2]. Behavior is often regarded as a sensitive sign of neural function, and maybe the ultimate assay since it embodies the integrity and integration of the nervous system [3]. Therefore, behavioral impacts may be as a result of either physical or pathologic changes that modifies nerve cell communication and integration [4].

Ethanol has been established as an agent capable of exerting dangerous effects on the central nervous system [5]. Patients and animal models have shown pathological changes in brain anatomy and neuronal function upon ethanol intoxication [2,6,7,8]. Significant harm is caused by ethanol in several distinct brain regions, including the frontal cortex, hippocampus, and cerebellum. Long-lasting impacts on emotional and memory impairments are caused by binge drinking, which may

indicate functional and structural changes in the hippocampus in teenage female mice [2]. Euphoria and disinhibition are the initial signs of acute intoxication, which develop into stupor and respiratory depression [9]. Abrupt cessation following extended or binge drinking can cause tremors, seizures, delirium tremens, severely constricted attentiveness, and fluctuating levels of alertness, agitation, and autonomic instability. This may be due to glutamate receptor up-regulation and GABA receptor down-regulation [9]. Ethanol also affects mental states as it has an impact on numerous neurotransmitter systems, favoring inhibitory γ-aminobutyric acid (GABA) receptors while inhibiting excitatory glutamate receptors [10]. For centuries, extracts from medicinal plant have demonstrated strong healing and health management potentials especially due to their anti-inflammatory, analgesic and anesthetic properties [11]. Tannic acid (TA) as a naturally occurring large polyphenol is known to demonstrate various medicinal capabilities such as being antioxidant [12] and neuroprotective [13]. This study carries out a neurobehavioral investigation of tannic acid treatments following ethanol-induced prefrontal cortex toxicity.

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 [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].

NEUROBEHAVIOURAL INVESTIGATION

This investigation was conducted on day 15 (24 hour after last treatment) to evaluate motor activities, anxiety, and cognitive trait variations amongst animals in all groups via carrying out an Open field test (OFT), an Elevated plus maze test, and a Y-maze test respectively**.**

Open field test (OFT)

OFT test is a common experimental measure of exploratory behaviour in rodents [17]. It is a test used to assay general locomotor activity levels, anxiety, and willingness to explore in animals (usually rodents) in scientific research [18]. The procedures used for this OFT were performed according to methods described by [19]. Motor coordination in adult wistar rats was assessed using the Open Field Test, specifically measuring Time Spent in the Center (TSC) and Time Spent in the Periphery (TSP) to evaluate behavioral responses following treatment with tannic acid after ethanol-induced prefrontal cortex toxicity.

Elevated Plus Maze Test

Elevated Plus Maze Test is a rodent model of anxiety that is used as a screening test for putative anxiolytic or anxiogenic compounds and as a general research tool in neurobiological anxiety research [20]. Each animal was placed in the centre of the maze, facing one of the open arms. It was allowed to explore the maze freely for a set duration, typically 5 minutes. The animal's behavior was recorded using a video recording system. Parameters measured include time spent in open arms (less time suggests higher anxiety), time spent in the closed arms, number of entries into each arm, number of entries into open and closed arms. The study measured the mean anxiety level in adult rats treated with tannic acid after ethanol-induced

prefrontal cortex toxicity, using the Elevated Plus Maze (EPM) test. The test evaluated anxiety-related behavior based on two key measures: Number of Open Arm Entries (NOAE) and Number of Closed Arm Entries (NCAE).

Y-Maze Test

The Y-maze test measures spatial working and recognition memory by making use of a rodent's natural exploratory instincts [21]. The animals were allowed to acclimate to the testing room for a specific period before testing (30 minutes). Each animal was placed at the end of one arm (usually designated as the start arm). It was allowed to explore the maze freely for a set duration, typically 5 minutes. A video recording system was set up to monitor and record the animals' behavior. In the context of the study, memory recognition was evaluated in adult Wistar rats using the Y-maze test, specifically assessing the mean percentage alternation as an indicator of cognitive function.

3. STATISTICAL ANALYSIS

Statistical analysis was carried out using IBM SPSS data analysis software version 26. All values that were generated out of this study were expressed as Mean ± Standard error of the Mean (SEM). Statistical difference in mean between groups were analyzed using one-way ANOVA (Analysis of variance), followed by t-test comparison of all groups. As there was a mean difference, a post-hoc test (Turkey) was carried out. P–value less than or equal to 0.05 was considered as statistically significant.

4. RESULTS

Table 1: Open Field Test

Group	TSC (sec)	TSP(sec)
A	2.50 ± 0.76	297.5 ± 0.76
B	3.33 ± 0.67	296.67± 0.67
C	6.83 ± 1.78	293.17 ± 1.78
D	3.33 ± 1.71	296.67 ± 1.71
E	1.17 ± 0.48	298.83 ± 0.48
F	4.00 ± 1.84	297.67 ± 1.23
P-value	0.11	0.05

Motor coordination was assessed in the rats using time spent in the center (TSC) and time spent in periphery (TSP). Results of the One-way ANOVA showed there was no statistically significant difference in both time spent in the center (TSC) and time spent in the periphery (TSP) ($F(5, 30) = 1.997$; $P = 0.11$), ($F(5, 30) = 2.525$, $p = 0.05$) respectively at $p<0.05$. The group C had the highest mean TSC (6.83 sec), indicating more exploratory behavior, while Group E had the lowest (1.17 sec). In terms of the TSP, this was relatively consistent across all groups, with Group E spending the most time in the periphery (298.83 sec) and Group C the least (293.17 sec). The TSP yielded a marginally significant result ($F(5, 30) = 2.525$, ($p= 0.05$).

Table 2: Elevated Plus Maze

Group	NOAE	NCAE
A	0.67 ± 0.33	1.83 ± 0.65
B	0.17 ± 0.17	1.50 ± 0.22
C	1.17 ± 0.40	1.33 ± 0.21
D	0.83 ± 0.40	1.17 ± 0.17
E	0.67 ± 0.33	2.67 ± 1.48
F	1.00 ± 0.26	1.17 ± 0.31
P-value	0.37	0.63

ISSN 2349-7823

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Anxiety was assessed in the rats using number of open arm entries (NOAE) and number of closed arm entries (NCAE). Results showed there was no statistically significant difference in the number of open arm entries (NOAE) ($F(5,30) = 1.12$; $P = 0.37$). Same is applicable in the NCAE which did not reveal a significant difference between groups ($F(5,30) = 0.70$; ($p= 0.63$).

Table 3: Y-maze Test

Group	% ALT
A	75.00 ± 17.08
B	60.56± 17.44
C	54.86 ± 18.52
D	51.67 ± 18.33
E	81.94 ± 9.23
F	49.21 ± 16.62
P value	0.66

Memory recognition was assessed in the rats using mean percentage alternation. Results from the one-way ANOVA indicated no statistically significant difference in mean percentage alternation between the treatment and control groups ($F(5, 30) = 0.65$; ($p= 0.66$). The p-value of 0.66, much higher than the significance threshold ($p < 0.05$),

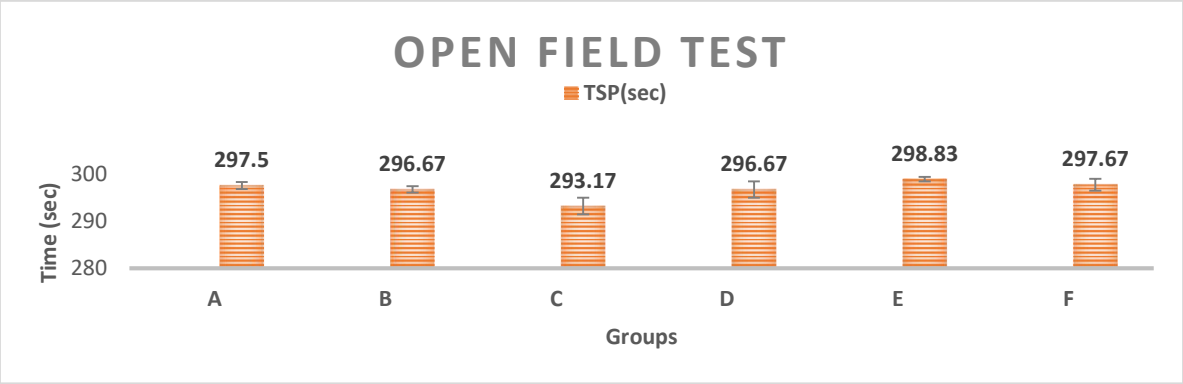

Figure 1: Graphical representation of the results from the time spent in periphery (TSP).

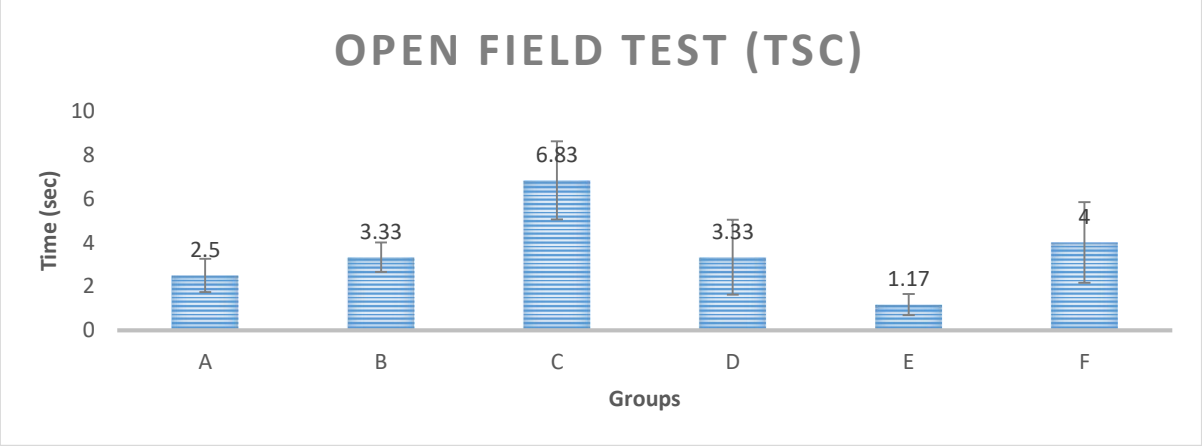

Figure 2: Graphical representation of the results from the time spent at the center (TSC)

ISSN 2349-7823

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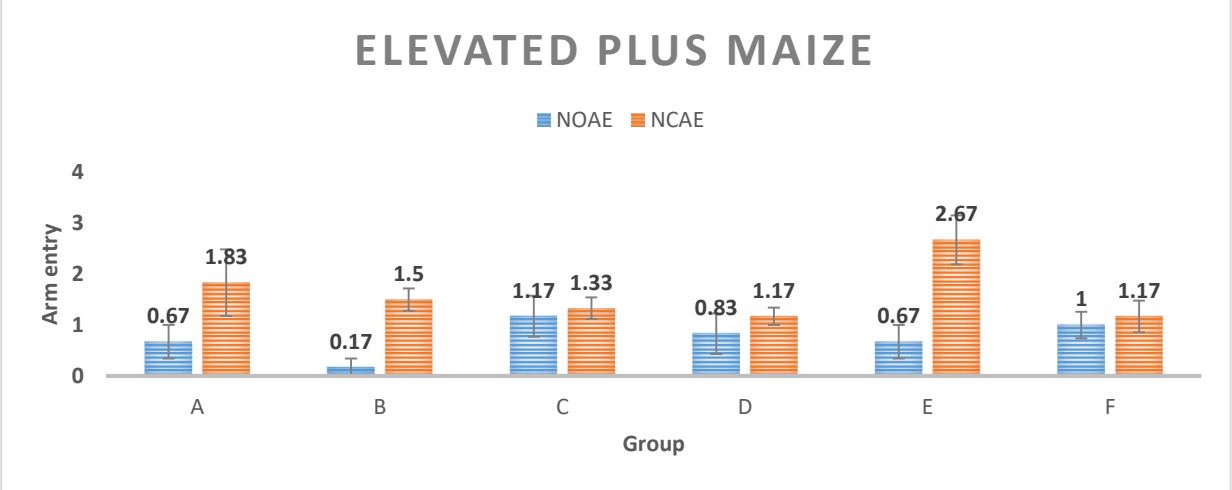

Figure 3: Graphical representation of the results from the Elevated Plus Maze Test displaying the number of open arm entries (NOAE) and number of closed arm entries (NCAE).

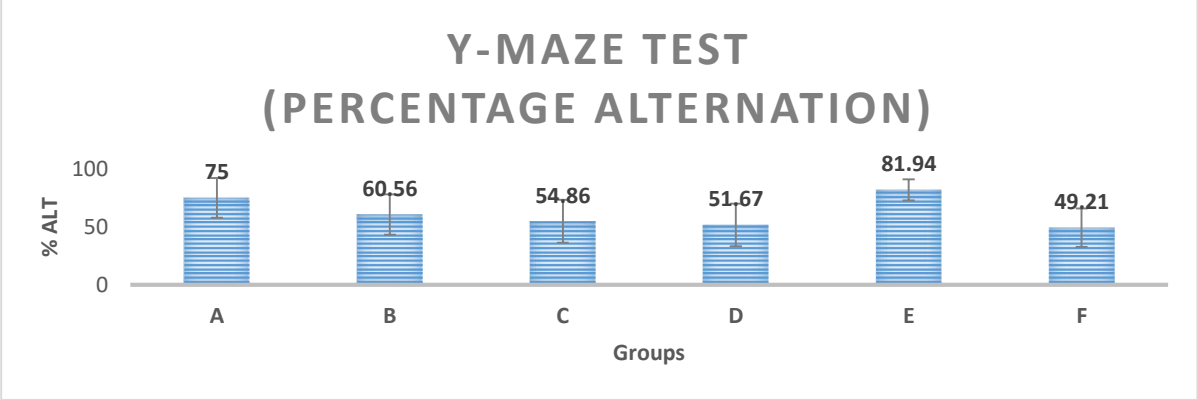

Figure 4: Graphical representation of the results from the Y-Maze Test displaying the percentage alterations.

5. DISCUSSION

Understanding the connection between the prefrontal cortex (PFC) and behavior can provide valuable insights into the neural mechanisms underlying various neurological and mental health conditions [4,22]. The PFC is involved in making decisions, holding and manipulating information in working memory, regulating emotions as well as impulsive behaviors including organizing and executing complex behaviors [23,24].

Our findings displayed alterations in the mean TSC and TSP among the groups, but these were not statistically significant. By implication, the TSC indicated no significant difference between groups, suggesting that the treatment had no significant effect on the rats' tendency to explore the center of the open field, which is often interpreted as reduced anxiety or increased exploratory behavior. The marginally significant result displayed on the TSP suggests that there might be slight differences in motor coordination or anxiety-related behavior, though this result is just at the threshold for statistical significance. These findings suggest that the treatment with tannic acid following ethanol-induced prefrontal cortex toxicity did not lead to significant improvements or deteriorations in motor coordination as measured by TSC and TSP. Also, the findings from the Elevated Plus Maze test suggest that tannic acid does not have a significant effect on anxiety-related behavior in rats, even after ethanol-induced prefrontal cortex toxicity. Both the NOAE and NCAE, which are key indicators of anxiety in this test, were not significantly different across the groups, indicating that tannic acid neither alleviated nor exacerbated anxiety levels in the rats. In addition, our findings also demonstrated that tannic acid did not notably affect memory recognition as measured by percentage alternation in the Y-maze test. Nevertheless, it is safe to hypothesize that tannic acid could potentially mitigate the effects of ethanol toxicity on brain regions like the prefrontal cortex, which are known to influence anxiety and decision-making. This is because previous reports have demonstrated that long-term post-treatment of TA exhibits protective effects against memory deficit and motor dysfunction [25] and another related study also reported that treatment with TA prevents memory deficits [26].

A systematic review on Pomegranate (Punicagranatum L.) and its phytochemicals as anxiolytic; TA from pomegranate were reported to posses' anxiolytic properties using animal's models [27]. The lack of significant differences between groups indicates that memory-related cognitive deficits induced by ethanol in the prefrontal cortex may not be substantially influenced by tannic acid treatment. Also the Y-maze test, which measures spontaneous alternation as a reflection of working memory and spatial recognition, showed that the mean percentage alternation remained comparable across all groups.

In general, the findings from this study suggest that although the doses of tannic acid used for treatment was not seen to significantly mitigate the behavioral or cognitive effects of ethanol-induced toxicity in these adult male Wistar rats; at least in terms of memory recognition or motor coordination, but also did not seem to cause significant harm or worsen the neurotoxic situation. Thus, higher doses may provide more reliable results. This is in agreement with a related study which reported that TA prevented memory deficits in streptozotocin-induced sporadic Alzheimer's disease; providing insights into memory, redox status, Na+, K+-ATPase and acetylcholinesterase activity [27]. Tannic acid (TA) was also reported to inhibit lipopolysaccharide-induced cognitive impairment in adult mice by targeting multiple pathological features [28]. Ashafaq et al., [29] also reported that tannic acid also modulated the behavioral deficits and neurodegeneration in experimental stroke challenged wistar rats.

6. CONCLUSION

Despite no significant behavioral improvements in motor coordination, anxiety levels and memory recognition levels, treatment with tannic acid demonstrated no additional adverse effects, maintaining the insights on its therapeutic potentials in higher doses following findings from other reports.

Conflict of interest

This study is not associated with any conflict of interest.

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