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MORPHOMETRIC EFFECTS OF TANNIC ACID TREATMENTS FOLLOWING ETHANOL-INDUCED NEUROTOXICITY

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Abstract: **Background: Alcohol abuse induces dangerous health conditions. Tannic acids (TA) are plant-based derivatives gaining attention in scientific research due to its medicinal potentials. Aim: To evaluate the morphometric effects of TA treatments following ethanol-induced neurotoxicity in adult male Wistar rats. 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. Morphometric study involved estimating total body weights, brain weights and brain volumes. Body weights were recorded at two days intervals. Brain weights and volumes were measured after animal sacrifice under anaesthesia, 24 hours after final treatment via weighing and fluid displacement in calibrated test tubes. Following statistical analysis, p–value≤0.05 was considered as statistically significant. Result: High-doses of TA demonstrated significant impact on body weights in the early stages of ethanol-induced toxicity. Brain weights analysis displays no statistically significant differences in brain weights among the various groups. Brain volumes analysis showed statistically significant differences between groups suggesting that TA improves brain volume in response to ethanol-induced damage. Conclusion: TA demonstrated positive morphometric and neuroprotective potentials.**

Keywords: **Tannic acid, Neuroprotective potentials, Neurotoxicity, morphometric, Wistar rats.**

1. INTRODUCTION

Alcoholism is a global problem. Overconsumption as well as abuse of alcohol can induce a variety of diseases and dangerous health conditions, such as alcoholic liver disease [1], chronic alcoholic encephalopathy [2] and neurocognitive disorders [3]. Alcoholics are often diagnosed with malnutrition as well as other nutrition deficiency health problems due to their high consumption of alcohol [4,5]. It has also been documented that due to the body's high demand for energy due to high alcohol consumption, their bodies preferentially use lipids as energy sources [4,6], resulting in an approximately 19% reduction in the adipose tissue component of alcoholic patients when compared to a healthy people; predisposing them to weight loss [4].

Ethanol has been reported to be a strong neurotoxic substance that can severely damage the normal function of the nervous system especially the brain [7]. Its neurotoxic mechanism can be linked to its induction of glutamate excitotoxicity, elevation of intracellular oxidative stress and inhibition of cell survival signals [8]. Ethanol initiates microglia activation along with the generation of inflammatory mediators, causing the dysfunction of the neural network; participating in many neurological

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and neurodegenerative diseases [9,10,11,12,13]. Alcohol-induced brain toxicity has also been associated with brain shrinkage demonstrated by a significantly reduced gray matter thickness [14] in addition to loss of total brain volume and cognitive decline [15,16]. Neuroimaging and postmortem research have confirmed the reduced brain weight in deceased alcoholic patients, along with effects associated with loss of gray and white matter [16,17].

Plants have been a source of medicinal substrates since the evolution of man. Plant-based chemicals have been used to cure many types of ailments [18]. Tannins are a class of naturally occurring polyphenol chemicals that are found in a wide range of trees and higher plant species, including coffee, green tea, and fresh fruits (pomegranate, persimmon, and grape) [19]. A few Clinical trials have demonstrated the efficacy of tannin-rich plant extracts, including green tea, grape seed phytopreparations, and polyphenol-rich chocolate [19]. The US Food and Drug Administration (FDA) has accepted tannic acid (TA), the most basic hydrolyzable tannin, as a food additive [20]. Due to its wide-ranging physiological effects (which include anti-inflammatory, anti-tumor, and anti-microbial properties) as well as its ability to interact with different proteins and its relevance to materials science and engineering, TA has garnered a lot of attention recently in the field of scientific research [20].

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 [21,22]. 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 [21,22]. 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 the positive control group [23].

Morphometric Study

The morphometric study involves estimating two variables: the total body weight of the experimental animals and their brain weights and volume. The total body weight of the animals was recorded eight consecutive times for all groups at two days apart until the end of the experiment using a digital weighing scale (ANTOM Electronic compact scale).

The animals were sacrificed using 60/30mg/kg/bwt of intraperitoneal ketamin/thiopental sodium as anaesthesia after 24 hours of fasting [24]. The skulls were dissected under anaesthasia and the brain tissues were quickly but carefully removed as a whole, washed with normal saline and then pat dry by an absorbent paper to remove excess moisture. Brain weights were recorded using a digital weighing scale while brain volumes were recorded via volume displacement in 10% formal saline in a calibrated test tube.

3. STATISTICAL ANALYSIS

Statistical analysis of all recorded values was carried out using the IBM SPSS data analysis software version 26. Descriptive analysis of brain weights, brain volumes and body weights of the various groups were done and their results reported as mean ± standard error of 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. Following 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.

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4. RESULTS

Descriptive results of Animal weights								
Group	day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15
A	185.67 ± 6.49 *c*	191.67 ± 6.88 *c*	195.67 ± 7.71	201 ± 7.24	204.5 ± 7.93	207.83 ± 8.86	210.5 ± 8.03	204.83 ± 6.24
B	196.5 ± 10.81	194.5 ± 11.75 *c*	189.5 ± 13.53	186.33 ± 14.2	174 ± 11.68	185.17 ± 13.63	158.83 ± 33.83	146.83 ± 31.36
C	232.83 ± 4.70 *f*	235.67 ± 3.59 *f*	211.5± 10.62	207.33 ± 10.13	213.5 ± 11.51	212.17 ± 11.61	207.5 ± 12.39	187.67 ± 11.07
D	221.67 ± 6.74	225.33 ± 6.39	213 ± 9.03	216.17 ± 12.16	209.83 ± 15.57	183.83 ± 38.41	180.17 ± 37.12	159.5 ± 33.62
E	202.33 ± 11.61	204.83 ± 12.38	213.83 ± 13.86	192.5 ± 14.94	190.67 ± 14.58	193 ± 14.70	192.33 ± 15.72	174.83 ± 13.51
F	189.83 ± 9.82	194 ± 7.85	192.67 ± 6.53	187.67 ± 5.35	189.83 ± 4.64	185.33 ± 4.70	181.17 ± 4.88	183 ± 7.37
Total	204.81 ± 4.35	207.67 ± 4.36	202.69 ± 4.36	198.5 ± 4.62	197.06 ± 4.96	194.56 ± 7.35	188.42 ± 8.98	176.11 ± 8.37
P-value	0.003	0.003	0.366	0.376	0.176	0.816	0.598	0.422
F stat	4.655	4.544	1.129	1.109	1.653	0.441	0.742	1.023
Key: "*c*" indicates significant difference from group C, "*f*" indicates significant difference from group F ($p < 0.05$)								

Table 1: Results of the One-way ANOVA showed there was a statistically significant difference in body weight at day 1 and 3 of the study at $p<0.05$ ($F(5, 30) = 4.655$; $P = 0.003$) ($F(5, 30) = 4.544$; $P = 0.003$). Post hoc test shows statistical significant difference between group A and C (0.008) and between group C and F (0.021) on day 1 at $p< 0.05$. It also shows a statistically significant difference between groups A and C (0.018), between groups B and C (0.033) and between groups C and F (0.030) on day 3 at $p< 0.05$. However, there was no statistically significant difference in body weight between groups at days 5, 7, 9, 11, 13 and 15 ($F(5, 30) = 1.129$; $P = 0.366$), ($F(5, 30) = 1.109$; $P = 0.376$), ($F(5, 30) = 1.653$; $P = 0.176$), ($F(5, 30) = 0.441$; $P = 0.816$), ($F(5, 30) = 0.742$; $P = 0.598$), ($F(5, 30) = 1.023$; $P = 0.422$) respectively at $p<0.05$.

Descriptive results of brain weights and volumes		
Group	Brain weight	Brain volume
A	1.73 ± 0.05	2.00 ± 0 *c,d*
B	1.70 ± 0.01	1.95 ± 0.03 *c,d*
C	1.68 ± 0.05	2.35 ± 0.09 *e,f*
D	1.80 ± 0.07	2.3 ± 0.08
E	1.73 ± 0.09	2.08 ± 0.05
F	1.63 ± 0.03	1.95 ± 0.05
Total	1.71 ± 0.03	2.10 ± 0.04
P-value	0.53	0
F stat	0.85	9.58

Table 2: Results of the One-way ANOVA showed there was no statistically significant difference in brain weight at $p<0.05$ ($F(5, 18) = 0.85$; $P = 0.53$). However, result of the One-way ANOVA showed a statistically significant difference in brain volume between groups ($F(5,18) = 9.58$; $P = 0.000$) at $p<0.05$. Post hoc test shows; a statistically significant difference for groups C (0.006) and D (0.025) compared to A, a statistically significant difference for group C (0.002) and D (0.006) compared to group B and a statistically significant difference for groups E (0.049) and F (0.02) compared to group C at $p <0.05$.

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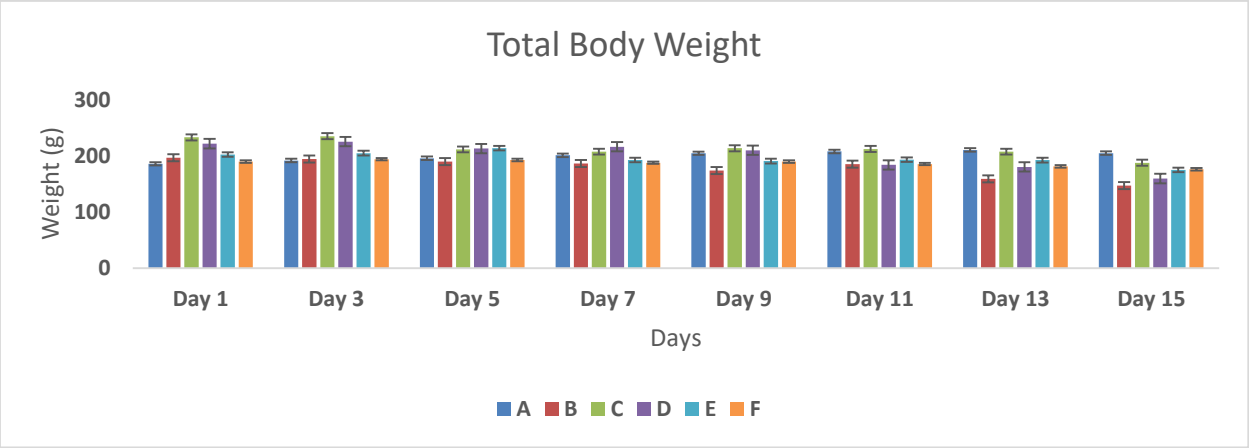

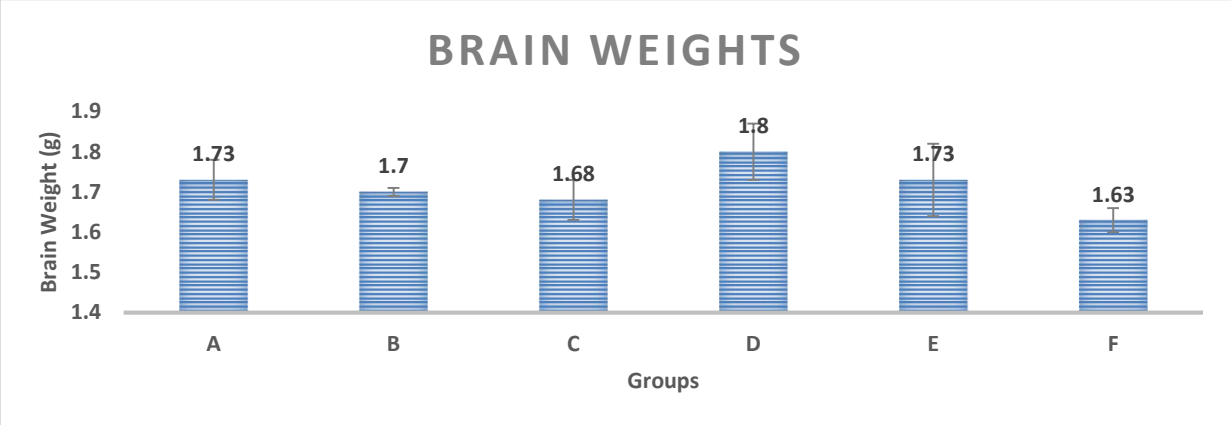

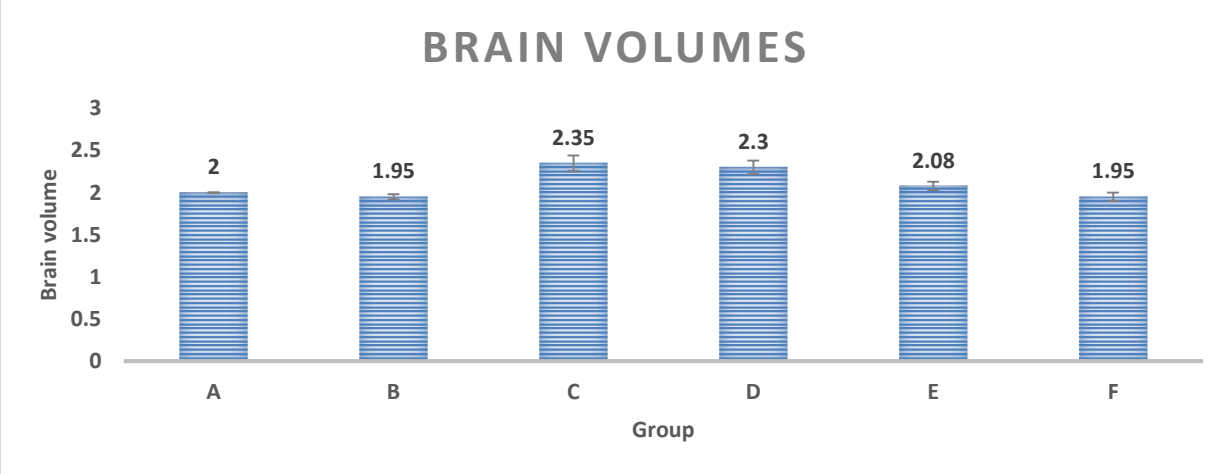

5. DISCUSSION

Body weights as well as brain weights and volumes analyses are a crucial aspects of animal research. As body weight analysis gives valuable understanding into a lot of physiological and pathological bodily processes, brain weights and volumes analyses make up a vital part of neuroscience research especially with regards to brain development, function, and disease [25,26,27]. These morphometric variables are indicators for toxicity assessments and adverse effects of various pharmacological interventions [28,29]. Significant alterations in brain volume can indicate neuroplasticity as well as adaptations in response to certain planned experiences, learning, or damage [26,28].

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The results suggest that tannic acid has a significant impact on body weights in the early stages (Days 1 and 3) of ethanol-induced toxicity. Specifically, high-dose tannic acid (Group C) seems to influence body weight differently compared to the control (Group A), ethanol-only (Group B), and vitamin E (Group F) treatments. The post-hoc analysis indicates that tannic acid might offer protective effects against the body weight loss typically seen in ethanol-induced toxicity, potentially through its antioxidant or anti-inflammatory properties.

However, the absence of significant differences in body weights from Day 5 onwards suggests that this initial impact of tannic acid may be transient. The rat's weight stabilizes, which might indicate that tannic acid's effects on metabolism or ethanol-induced weight changes are more pronounced in the early stages of exposure and treatment. These findings could imply that tannic acid helps mitigate the acute toxic effects of ethanol on metabolism or body weight in the short term but does not lead to long-term changes in body weight once the initial stress response to ethanol is managed. This also suggests that tannic acid could play a role in early intervention against ethanol-induced damage but may not significantly affect chronic weight outcomes.

The findings from the brain weight analysis displays no statistically significant differences in brain weights among the various groups ($F(5,18) = 0.85$; ($p = 0.53$), indicating that ethanol exposure and tannic acid treatments did not significantly impact overall brain weight. However, brain volumes showed statistically significant differences between groups ($F(5,18) = 9.58$; ($p= 0.000$). The post hoc analysis revealed that, groups C (high-dose tannic acid) and D (medium-dose tannic acid) showed statistically significant differences in brain volumes compared to group A (normal control) and group B (ethanol-only).Groups E (low-dose tannic acid) and F (vitamin E) also had statistically significant differences compared to group C. On the effect of tannic acid on brain volumes, the significant increase in brain volumes for the high-dose (C) and medium-dose (D) tannic acid groups compared to the ethanol-only (B) and control groups (A) suggests that tannic acid helps preserve or improve brain volume in response to ethanol-induced damage. This preservation may indicate that tannic acid offers some neuroprotective effects, counteracting ethanol's potential neurotoxic impact, which can lead to brain atrophy or reduced brain volume as reported in previous studies [12,14,27,31]. These neuroprotective potentials attributed to the treatments with tannic acid can be backed up by previous reports on the neuroprotective capabilities of tannic acids [32] which has been attributed to its antioxidant, anti-inflammatory and antiapoptotic properties [33,34,35].

6. CONCLUSION

The significant findings regarding brain volume suggest that tannic acid may play a crucial role in counteracting ethanol's neurotoxic impact. While the initial protective effects on body weight indicate potential early intervention benefits, the lack of sustained weight changes suggests that further investigation into the long-term effects of tannic acid is warranted.

Conflict of interest

This study is not associated with any conflict of interest.

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