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Original Research Article

Theaflavin Alleviates Memory Deficit and Anxiety-Like Phenotypes in Valproic Acid Murine Model of Autism: Impact on Cholinergic and Oxido-Nitrigic Stress Mechanisms

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ABSTRACT

Autism Spectrum Disorder (ASD) is a neurodevelopmental condition marked by deficits in communication and repetitive behaviors. Its etiology is multifactorial, involving genetic, epigenetic, and environmental factors, complicating diagnosis and treatment. This study explores the neuroprotective effects of theaflavin (TF) in a valproic acid (VPA)-induced murine model of autism, focusing on its impact on anxiety, memory, oxidative-nitric stress, and cholinergic pathways. Pregnant Swiss albino mice were injected with VPA (250 mg/kg) on gestational day 13. Male offspring, confirmed to exhibit autistic-like behaviors through tests such as Y-maze, NORT, EPM, LDT, and HBT, were treated with TF (10 or 20 mg/kg) or 1% DMSO (10 mL/kg) for 30 days. Behavioral assessments were repeated post-treatment, and brain samples were analyzed for oxidative stress markers (NO, GSH) and histological damage in the medial prefrontal cortex and hippocampus. VPA-exposed mice showed impaired memory, increased AChE and NO levels, reduced GSH, and neuronal damage. TF significantly improved memory in the Y-maze [$F(3, 16) = 9.448$; ($p = 0.0008$)] and NORT [$F(3, 16) = 15.12$; ($p < 0.0001$)] but did not significantly affect anxiety-like behaviors in EPM, LDT, or HBT. TF treatment reduced oxidative stress, mitigated neuronal damage, and enhanced cholinergic activity, highlighting its potential as a therapeutic agent for autism.

Keywords: Theaflavin, Autism, Anxiety, Memory, Oxidative Stress.

Introduction

Autism Spectrum Disorder (ASD) is a complex neurodevelopmental condition characterised by challenges in social interaction, communication difficulties, and restricted or repetitive behaviours.[1-3] The aetiology of ASD is multifaceted, involving genetic, epigenetic, and environmental factors, which complicates both diagnosis and treatment.[4,5] Among various proposed environmental contributors to ASD, prenatal exposure to teratogenic substances such as valproic acid (VPA) has been extensively studied. Valproic acid is a well-established anticonvulsant drug that, when administered during pregnancy, has been shown to significantly increase the risk of ASD in offspring, potentially by disrupting neurodevelopmental processes.[6-8] The murine model of autism induced by VPA has gained considerable attention in recent years due to its ability to mimic several behavioural and neurobiological characteristics of autism in humans.[9]

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These characteristics include anxiety-like behaviours, impairments in social interaction, and cognitive deficits, making it a valuable tool for evaluating therapeutic interventions.[10] Therefore, identifying potential treatments that can alleviate these symptoms is of utmost importance for enhancing the quality of life for affected individuals and their families. Plant-based compounds have shown promising effects in the management of CNS-related disorders. Hence, theaflavin, a polyphenolic compound which is primarily found in black tea[11] a product of catechins oxidation during the fermentation of black tea, has been shown to exert various neuroprotective effects, partly due to their antioxidant, anti-inflammatory, and neurogenic properties.[12] Moreover, emerging evidence suggests that theaflavins may exert their effect by modulating oxidative stress mechanisms, which may be dysregulated in several CNS-related disorders.[13] Also, the potential impact of theaflavin in mitigating neuroinflammation associated with the VPA-induced murine model of autism presents a unique avenue for research. As oxidative stress and neuroinflammation are potential culprits implicated in the pathophysiology of autism[14], exploring the neurobiological pathways affected by theaflavin may yield insights into novel therapeutic strategies. Furthermore, understanding the mechanisms through which theaflavin exerts its effects can help elucidate the pathophysiological underpinnings of the behavioural deficits as seen in autism, thereby contributing to a more comprehensive understanding of the disorder.

Materials and Methods

Experimental Animal

Pregnant Swiss albino mice, aged 12±1.5 weeks and weighing 30–35 g, were utilised for this study. They were housed in plastic cages under standard room temperature conditions, following a 12-hour light/dark

cycle in the animal facility of the Faculty of Basic Medical Science, Delta State University, Abraka. The mice were provided with a balanced rodent pellet diet and had access to water freely. On gestation day 13, pregnant female mice received an intraperitoneal (i.p.) injection of 250 mg/kg VPA. Male offspring were then allocated into four groups (n = 5). The mice were acclimated for at least two weeks before starting the experiments. Ethical approval was granted by the Faculty's Research and Ethics Committee of the Basic Medical Science, Delta State University, Abraka, Nigeria (RBC/FBMC/DELSU/24/328), and all procedures adhered to the NIH Guidelines for the Care and Use of Laboratory Animals.

Drugs and Chemicals

5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) from Aldrich, Germany; trichloroacetic acid (TCA) from Burgoyne Burbidges & Co., Mumbai, India; thiobarbituric acid (TBA) from Guanghua Chemical Factory Co. Ltd., China; Tris (hydroxymethyl)-aminomethane (Tris buffer) from Hopkin & Williams Company, USA; acetic acid from Sigma-Aldrich, Inc., St. Louis, USA; $NaHCO_3$ from BDH Chemicals Ltd., Poole, England; sodium carbonate from Fisons, Loughborough, Leics, England; $Na_2HPO_4 \cdot H_2O$, $NaH_2PO_4 \cdot H_2O$, K_2HPO_4, $K_2Cr_2O_7$, and KCl, all from BDH Chemicals Ltd., Poole, England; NaOH from JT Baker Chemicals Co., Phillipsburg, NJ, USA; dimethyl sulfoxide (DMSO); theaflavin (at doses of 10 mg/kg and 20 mg/kg); valproic acid (250 mg/kg); and ethanol.

Drug Preparation and Treatment

Theaflavin (TF) was prepared by dissolving 350 mg in 35 mL of 1 % dimethyl sulphoxide (DMSO) to obtain the stock solution. The stock solution was further diluted with distilled water to get the concentration used in the study. Also, the other drug used in this study was dissolved in distilled water before use. On gestational day 13, female pregnant mice received valproic acid (VA) intraperitoneally at 250mg/kg. Male mice were randomly allotted into 4 groups. Group 1 (pups from pregnant female mice), the normal control, received vehicle 10 mL/kg (1 % DMSO). Male offspring with autistic phenotype were further allotted into groups 2-4. Group 2 served as the pathologic group, while groups 3 and 4 received 10 mL/kg (1 % DMSO), theaflavin (TF) at a dose of 10mk/kg, 20mg/kg, respectively from postnatal day 22 to postnatal day 51. The dose of TF used in this study was selected based on the results obtained from pilot studies carried out in the lab, while that of valproic acid was in line with the other literatures.[15]

Behavioural Assays

Assessment of memory performance using the Y-Maze

Memory function altercation was evaluated using the Y-maze spatial working memory task as previously described by [16] with little modifications. The Y-maze apparatus comprises three equally spaced arm chambers (120°, 41 cm long, 15 cm high and 5cm wide) labelled alphabetically: A, B and C. Each mouse underwent an exploration test. Each was placed into arm A of the apparatus and allowed to move freely. That is, explore the three chambers for 5 min without reinforcement. An arm entry was scored when the four paws of the animals were completely in the arm of the Y-maze, while alternation was measured when an animal entered into all three arms in consecutive order. Percentage alternation (an index of spatial working memory) was then calculated using equation 1:

$$\text{Index of spatial working memory } \frac{\text{Total alternation number}}{\text{Total number of entries} - 2} X\ 100 \quad \textbf{(1)}$$

Novel Object Recognition Test

The effect of theaflavin on memory performance was also tested using the Novel Object Recognition test (NORT) in an open-field chamber (60 cm × 50 cm × 40 cm) as described earlier.[17] This test consists of two phases: the pre-test phase and the test phase. The pre-test phase involves placing the experimental mice at point zero (0) in between two identical objects (A and B) placed at opposite sides of the open-field chamber, 8 cm (from the walls) and 34 cm (from each other) for 5 min. The duration the mouse spent (s) exploring each object was recorded. The animal was then returned to its home cage and rested for about 2 hrs. For the test phase, object B was replaced with object C (an object not identical to objects A and B). The mice were left to explore objects A and C for about 5 min, and the time each mouse spent (s) exploring each object was then recorded. The working apparatus was cleaned with 10% ethanol after each test. The discrimination index, which is used as a measure of nonspatial/recognition memory function, will be calculated using Equation 2

$$\frac{\text{Time spent exploring novel object} - \text{time spent exploring familiar object.}}{\textbf{\textit{Total time spent exploring both objects}}} X\ 100 \quad \textbf{(2)}$$

Assessment of Anxiety Levels Using Elevated Plus Maze Test

Elevated plus maze (EPM): an accepted test for anxiolytic and anxiogenic effects of a drug. This test was utilised to measure the possible anxiety-like alterative effects of theaflavin in experimental mice using the procedure described by [18] with little modifications. Briefly, the plus maze apparatus consists of two open arms and two closed arms and an elevation of 25 cm above the floor level. Each mouse was placed at the tip or end of an open arm, with the head pointing towards the centre of the apparatus. The experimental animal was allowed to explore the maze for 5 min, during which the number of arm entries (either open or closed) and the time spent in open and closed arms were noted and recorded.

Assessment of Anxiety Levels Using Light /Dark Box

The light/dark transition test was utilised to further justify the possible anxiolytic property of the aflavin using the method of.[18] Briefly, the apparatus consisted of a rectangular box (45 × 27 × 27 cm), partitioned into two compartments connected by a 7.5 × 7.5 cm opening in the wall between the two compartments. The mouse was dropped in the illuminated compartment of the box, and the number of entries and time spent in the light and dark compartments of the box were taken and recorded within a 5-minute session.

Effect of TF on Anxiety-Like Behaviour using the Hole Board Test

The Hole Board Test was used to assess anxiety-like behaviour in mice according to a previously described method.[19] The test involves placing the mice on an elevated board with evenly spaced holes and observing their behaviour for 5 minutes. Key behaviours measured include head dips (indicating exploration). Latency to the first head dip was observed and analysed.

Biochemical Assays

After testing for memory and anxiety function, the mice were euthanised, and their brains were harvested and kept in the refrigerator with an ice block for 30 min. After that, the whole brain was weighed, sectioned and homogenised with 10% w/v phosphate buffer (0.1 M, pH 7.4). Each brain tissue homogenate was separated into various portions for different biochemical assays.

Determination of Acetylcholinesterase (ACHE) Activity in Mice Brain

Aliquots of homogenates of the individual mouse brains of the various treatment groups were taken and used to measure AChE activity, a marker for cholinergic neurotransmission.[20] Briefly, AChE activity in the homogenate was measured by adding 2.6 mL of phosphate buffer (0.1 M, pH 7.4), 0.1 mL of 5,5′-dithiol-bis (2-nitrobenzoic acid) (DTNB) and 0.4 mL of the homogenate. Then, 0.1 mL of acetylthiocholine iodide was added to the reaction mixture. The absorbance was read using a spectrophotometer (Agilent Cary 60 UV-Vis United States) at a wavelength of 412 nm, and a change in absorbance for 10 min at two-minute intervals was recorded. The rate of AChE activity was measured by following the increase of colour produced from thiocholine when it reacts with DTNB. The change in absorbance per minute was determined, and the rate of AChE activity was calculated and expressed as μmol/min/g tissue.

Determination of glutathione (GSH) Concentration

Aliquots of brain homogenates of individual mice in the respective treatment groups were taken, and the GSH concentration was

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determined using the method of Moron as adopted by.[21] Equal volumes (0.4 mL) of brain homogenate and 20% TCA (0.4 mL) was mixed and then centrifuged using a cold centrifuge at 10,000 rpm at 4 °C for 20min. The supernatant (0.25 mL) was added to 2 mL of 0.6 mMDTNB, and the final volume was made up to 3 mL with phosphate buffer (0.2M, pH 8.0). The absorbance was then read at 412 nm against a blank reagent using a spectrophotometer. The concentrations of GSH in the brain tissues were expressed as micromoles per gram tissue (µmol/g tissue).

Estimation of Brain Nitrite Level

Brain nitrite concentration was estimated using Greiss reagent, which serves as an indicator of nitric oxide production. 100 µL of Greiss reagent (1:1 solution of 1% sulfanilamide in 5% phosphoric acid and 0.1% of N-1-naphthyl ethylenediamine dihydrochloride) was added to 100 µL of the supernatant and absorbance was measured at 540 nm.[22] The brain nitrite concentration was estimated using a standard curve of sodium nitrite (0-100 uM).

Statistical Analysis

All data were presented as Mean ±SEM. The results were analysed by one-way analysis of variance (ANOVA), and posthoc tests (Tukey test) were carried out to determine the source of significant main effect using Graph Pad InStat® Biostatistics software version 10.3.0. The level of significance for all tests was set at. P value was set at < 0.05.

Results and Discussion

The effect of Theaflavin (TF) given daily for 30 days on memory performance, as measured by the percentage of alternation in the Y-maze test and novel object recognition test (NORT), are shown in **Figures 1** and 2, respectively. The results showed that there were significant differences between treatment groups. Posthoc analysis using Tukey's posthoc test revealed that the VPA group showed a significant (p<0.05) reduction in memory performance as depicted by a reduced percentage alternation compared to the control group. Meanwhile, TF (10, 20 mg/kg) given daily for 30 days produced a significant (p<0.05) increase in memory performance, as evidenced by an increased percentage of alternation when compared with the valproic acid (VPA) group. Specifically, TF at 20 mg/kg showed the most significant (p<0.05) improvement compared to the VPA group. The effect of TF given daily for 30 days on recognition memory, as measured by the discrimination index, is shown in Figure 2. One-way ANOVA showed that there were significant differences between treatment groups. Posthoc analysis using Tukey's posthoc test revealed that the VPA group showed a significant (p<0.05) reduction in preference for the novel object, as depicted by a reduced discrimination index compared to the control group. In contrast, TF (10, 20 mg/kg) given daily for 30 days produced a significant (p<0.05) increase in preference for the novel object, as evidenced by increased discrimination indices when compared with the valproic acid (VPA) group.

The effect of Theaflavin (TF) 30 days administration on anxiety-like behaviours, as measured by the time spent in the closed arm as seen in the elevated plus maze (EPM), as time spent in the dark box as seen in the light/dark transition box, and the number of head dips as seen in the hole board test are shown in Figures 3, 4 and 5, respectively. One-way ANOVA showed that there were no significant differences between treatment groups. Posthoc analysis using Tukey's post hoc test revealed that TF (10, 20 mg/kg) given daily for 30 days produced no significant (p<0.05) effect when compared to the VPA-treated group.

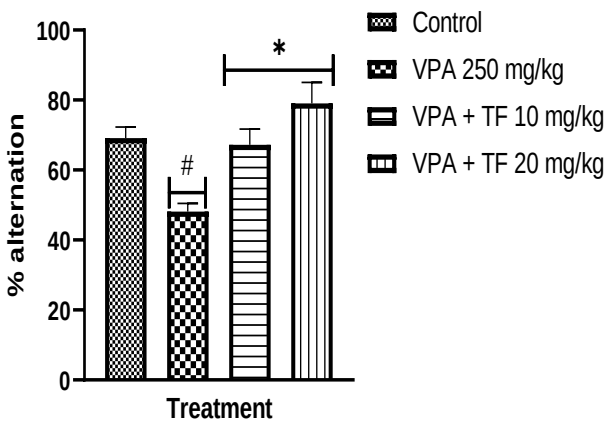

Figure 1: The effect of theaflavin on memory performance in prenatal valproic acid murine model using the Y-maze.
Values represent the mean ± SEM for 5 animals per group
# p<0.05 compared to the control group (ANOVA followed by Turkey's posthoc test).
p<0.05 compared to the pathologic group (ANOVA followed by Turkey's posthoc test).
VPA: Valproic acid, TF: Theaflavin

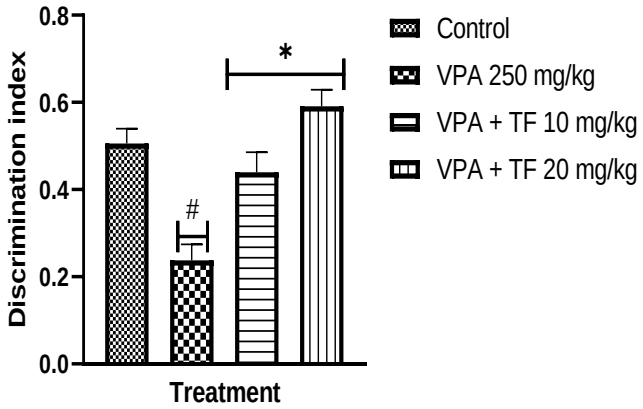

Figure 2: The effect of theaflavin on memory performance in prenatal valproic acid murine model using the novel object recognition test (NORT).
Values represent the mean ± SEM for 5 animals per group
# p<0.05 compared to the control group (ANOVA followed by Tukey's posthoc test).
p<0.05 compared to the pathologic group (ANOVA followed by Tukey's posthoc test).
VPA: Valproic acid, TF: Theaflavin

Similarly, the effect of Theaflavin (TF) given daily for 30 days on acetylcholine esterase levels in the prefrontal cortex and hippocampus is shown in Figure 6. One-way ANOVA showed significant differences between treatment groups in the prefrontal cortex. Posthoc analysis using Tukey's posthoc test revealed that the VPA group showed a significant (p<0.05) increase in acetylcholine esterase levels when compared to the control group, while TF (10, 20 mg/kg) given daily for 30 days produced a significant (p<0.05) decrease in acetylcholine esterase levels when compared with the valproic acid (VPA) group. Specifically, TF at 20 mg/kg showed the most significant (p<0.05) decrease compared to the VPA group.

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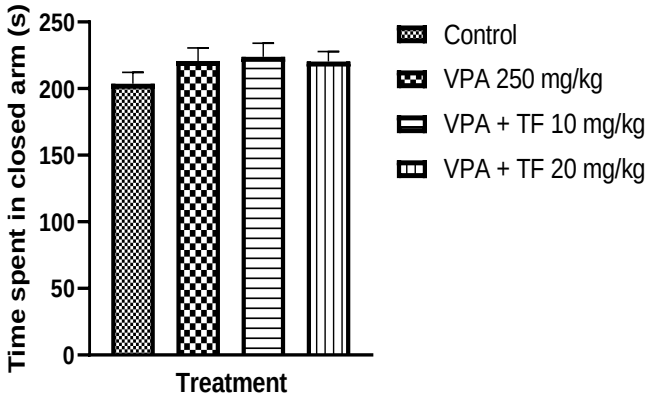

Figure 3: The effect of theaflavin on anxiety-like behaviour in a prenatal valproic acid murine model using the elevated plus maze (EPM).
Values represent the mean ± SEM for 5 animals per group
p>0.05 compared to the control group (ANOVA followed by Tukey's posthoc test).
p>0.05 compared to the pathologic group (ANOVA followed by Tukey's posthoc test).
VPA: Valproic acid, TF: Theaflavin

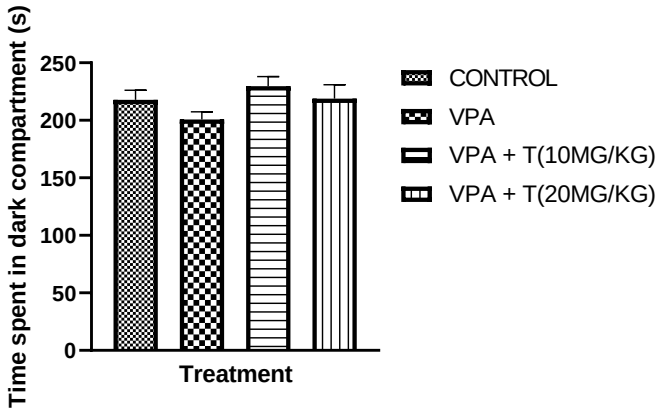

Figure 4: The effect of theaflavin on anxiety-like behaviour in a prenatal valproic acid murine model using the light and dark transition box**.**
Values represent the mean ± SEM for 5 animals per group
p>0.05 compared to the control group (ANOVA followed by Tukey's posthoc test).
p>0.05 compared to the pathologic

One-way ANOVA showed significant differences between treatment groups in the hippocampus. Posthoc analysis using Tukey's posthoc test revealed that the VPA group showed a significant (p<0.05) increase in acetylcholine esterase levels when compared to the control group, while TF (10, 20 mg/kg) given daily for 30 days produced a significant (p<0.05) decrease in acetylcholine esterase levels when compared with valproic acid (VPA) group. Specifically, TF at 10 mg/kg showed the most significant (p<0.05) decrease compared to the VPA group.
Furthermore, the effect of 30 days of administration of Theaflavin (TF) on antioxidant activity in the prefrontal cortex and hippocampus is shown in Figure 7. One-way ANOVA showed significant differences between treatment groups in glutathione levels in the prefrontal cortex and hippocampus. Posthoc analysis using Tukey's post hoc test revealed that the VPA group showed a significant (p<0.05) decrease in glutathione levels when compared to the control group, while TF (10, 20 mg/kg) given daily for 30 days produced a significant (p<0.05) increase in glutathione levels when compared with the valproic acid (VPA) group. Also, the effect of Theaflavin (TF) given daily for 30 days on nitric oxide activity in the prefrontal cortex and hippocampus is

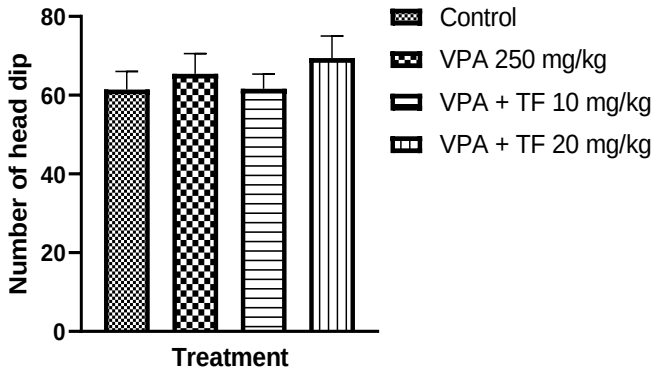

Figure 5: The effect of theaflavin on anxiety-like behaviour in a prenatal valproic acid murine model using the hole board test.
Values represent the mean ± SEM for 5 animals per group
p>0.05 compared to the control group (ANOVA followed by Tukey's posthoc test).
p>0.05 compared to the pathologic group (ANOVA followed by Tukey's posthoc test).
VPA: Valproic acid, TF: Theaflavin

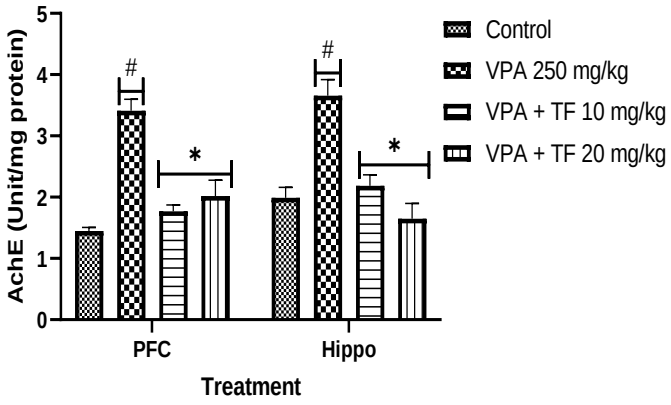

Figure 6: The effect of theaflavin on acetylcholinesterase levels in prenatal valproic acid murine model.
Values represent the mean ± SEM for 5 animals per group
# p<0.05 compared to the control group (ANOVA followed by Tukey's posthoc test).
p<0.05 compared to the pathologic group (ANOVA followed by Tukey's posthoc test).
VPA: Valproic acid, TF: Theaflavin

shown in Figure 8. One-way ANOVA showed significant differences between treatment groups in nitric oxide levels in the prefrontal cortex and hippocampus. Posthoc analysis using Tukey's posthoc test revealed that the VPA group showed a significant (p<0.05) increase in nitric oxide levels when compared to the control group, while TF (10, 20 mg/kg) given daily for 30 days produced a significant (p<0.05) decrease in nitric oxide levels when compared with the valproic acid (VPA) group (Figure 8).
The effect of theaflavin on the histology and neuronal density count of the medial prefrontal cortex (mPFC) in prenatal valproic acid murine model and on the histology and neuronal density count of the cornu ammonis (CA2) of the hippocampi in prenatal valproic acid were also investigated. The findings demonstrated that theaflavin (TF) improved spatial working memory in mice by increasing their alternation behaviour in the Y-maze test. Additionally, TF significantly reversed the memory impairment caused by VPA, as indicated by the reduction in percentage alternation in the Y-maze test. The Y-maze test, a widely used animal model, is often employed to assess the memory-enhancing potential of pharmacological agents in rodents.[23]

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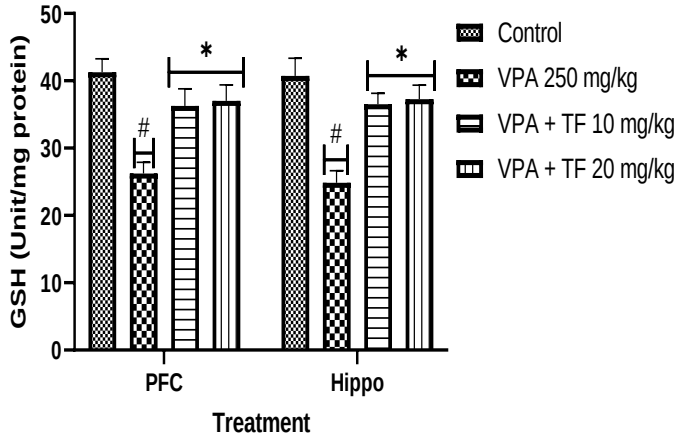

Figure 7: The effect of theaflavin on glutathione levels in prenatal valproic acid murine model.
Values represent the mean ± SEM for 5 animals per group
# p<0.05 compared to the control group (ANOVA followed by Tukey's posthoc test).
p<0.05 compared to the pathologic group (ANOVA followed by Tukey's posthoc test).
VPA: Valproic acid, TF: Theaflavin

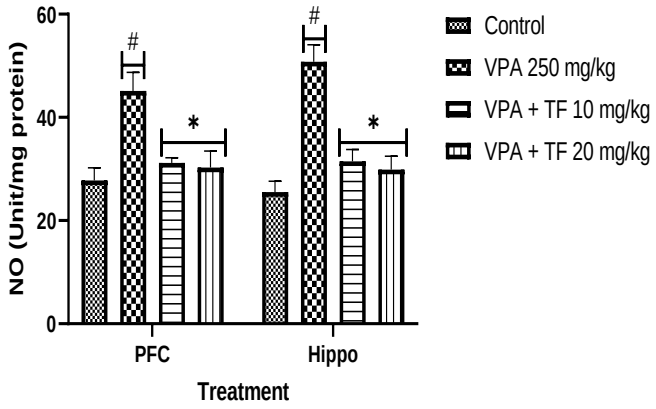

Figure 8: The effect of theaflavin on nitric oxide levels in prenatal valproic acid murine model.
Values represent the mean ± SEM for 5 animals per group
# p<0.05 compared to the control group (ANOVA followed by Tukey's posthoc test).
p<0.05 compared to the pathologic group (ANOVA followed by Tukey's posthoc test)
VPA: Valproic acid, TF: Theaflavin

This test relies on the mouse's ability to navigate the Y-maze arms in a specific sequence, known as spontaneous alternations, which reflects their spatial working memory. By remembering the last arm visited, rodents avoid revisiting the same arm, serving as a measure of short-term memory.[23] TF's ability to increase alternation behaviour suggests that it may enhance memory in the VPA-induced autism model in mice. The impact of TF on memory performance was also examined in mice using the novel object recognition test (NORT), which evaluates nonspatial working memory by taking advantage of mice's natural curiosity to explore their environment.[24] The NORT is a standard tool for assessing memory function, relying on rodents' inherent preference for novel objects.[25] A preference for a new object over a familiar one indicates that the animal remembers the familiar object.[24] Memory performance in this test is gauged by comparing the time spent exploring the novel object versus the familiar one, with the level of

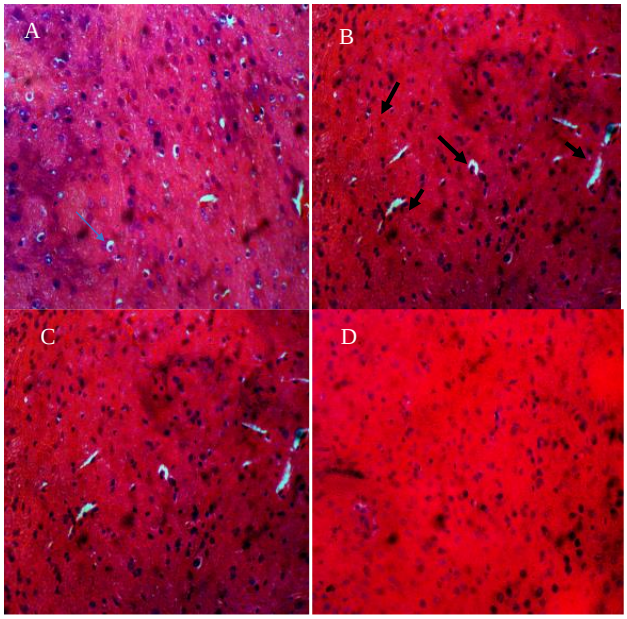

Figure 9a. Representative photomicrograph (HandE-stained section) of the effect of theaflavin on the medial prefrontal cortex (mPFC) in a prenatal valproic acid murine model.
Magnification = HE × 400. A= Control, B= VPA, C= VPA+ TF 10mg/kg, D= VPA+ TF 20mg/kg slide A revealed that there is no observable lesion, slide B reveals atrophy of the neurons, slide C reveals no observable lesion, slide D reveals no observable lesion

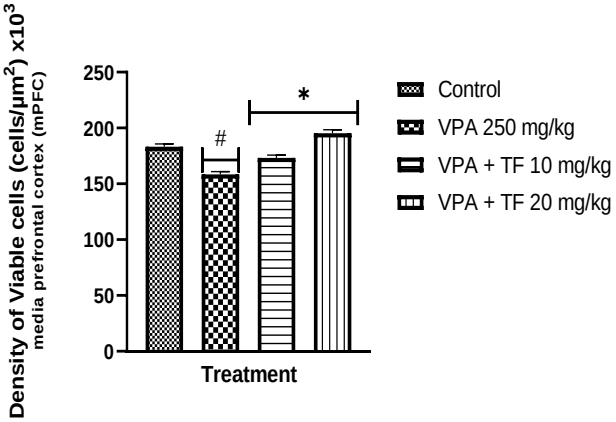

Figure 9b: The effect of theaflavin on the histology and neuronal density count of the medial prefrontal cortex (mPFC) in prenatal valproic acid murine model.
Values represent the mean ± SEM for 5 animals per group
# p<0.05 compared to the control group (ANOVA followed by Tukey's posthoc test).
* p<0.05 compared to the pathologic group (ANOVA followed by Tukey's posthoc test).
VPA: Valproic acid, TF: Theaflavin

exploration depending on how well the familiar object is remembered.[24,26-29] In this study, VPA reduced the time spent exploring the novel object, suggesting impaired memory. However, TF significantly counteracted the VPA-induced memory impairment, as evidenced by the increased preference for the novel object, indicating its potential beneficial effect on memory deficits linked to autism.
The impact of TF on anxiety-like behaviour was further assessed using the elevated plus maze (EPM), which leverages rodents' natural

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aversion to open areas and their preference for enclosed spaces. Typically, anxious animals will spend more time in the closed arms and less in the open arms of the maze.[30] In this study, TF treatment did not significantly affect the time spent in either the open or closed arms, indicating no substantial effect on anxiety after VPA administration. Similarly, in the light and dark transition box (LDT), which evaluates rodents' preference for dark, enclosed spaces over brightly lit, open areas [31], no significant changes in time spent in either compartment were observed following TF treatment. This further suggests that TF had minimal impact on anxiety-related behaviour. Additionally, the hole board test (HBT), which measures anxiety and exploratory behaviour based on head-dipping activities [32-33], revealed no significant changes in the frequency or duration of head-dipping after TF administration. These findings indicate that TF had little effect on anxiety-like behaviour at the administered doses in rodents subjected to the VPA model of autism. The pharmacological assessment of anxiety and memory functionality typically includes both behavioural and biochemical approaches. The biochemical approach evaluates the pathological processes that may impact the behavioural aspects of memory and anxiety. In this study, biochemical assays showed that TF exhibited anti-cholinesterase and antioxidant effects in mice brains. Inhibition of acetylcholinesterase activity results in increased levels of acetylcholine (ACh) in the brain. Cholinergic neurotransmission is essential for memory and learning, and drugs that facilitate cholinergic transmission often enhance cognitive functions.[34] This study found that treatment with TF (10 mg/kg, 20 m/kg) significantly decreased AChE activity in the prefrontal cortex and hippocampus, which are core brain regions implicated in cognition (memory and learning) compared to the VPA-treated group. The finding suggests that TF inhibition of AChE activity plays an ameliorative role by reducing acetylcholine hydrolysis and enhancing cholinergic neurotransmission, which is seen to be defective in autism.

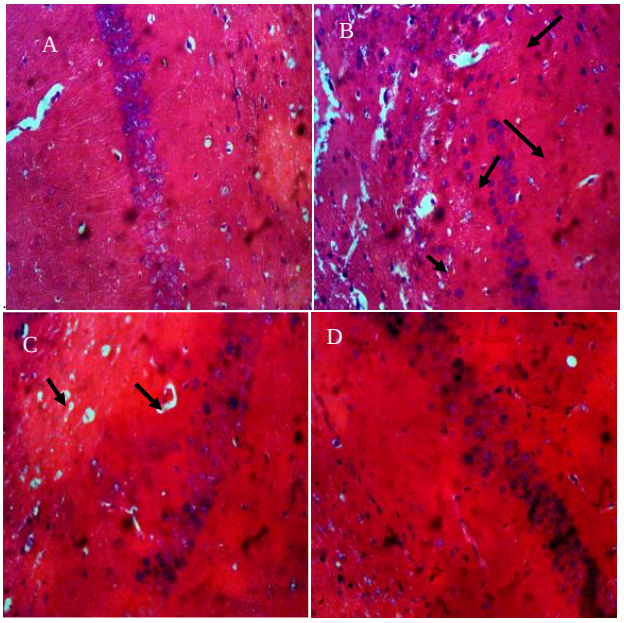

Figure 10a. Representative photomicrograph (H&E-stained section) of the effect of theaflavin on the CA2 region of the hippocampus in a prenatal valproic acid murine model.
Magnification = HE × 400. A= Control, B= VPA, C= VPA+ TF 10mg/kg, D= VPA+ TF 20mg/kg slide A revealed that there is no observable lesion, slide B reveals There is necrosis and loss of neurons, slide C reveals acute degeneration and pyknosis of neurons, and slide D reveals no observable lesion

In line with previous studies, VPA-treated mice showed an increased level of oxidative stress in both the prefrontal cortex and hippocampus.[35-38] This stress results in an imbalance between prooxidant and antioxidant levels in the body. This study showed an increased NO level in brain tissues, including the prefrontal cortex and hippocampus. Similarly, GSH levels were reduced in the VPA-treated group compared to controls. However, TF reduced brain oxidative stress by increasing the levels of GSH in the prefrontal cortex and hippocampus and reducing NO levels in these brain regions. The result from this study shows that VPA causes necrosis and a loss of neurons in the mPFC and CA2 region of the hippocampus, which play crucial roles in memory and social recognition. This is consistent with other studies showing that VPA induces excitotoxicity and oxidative damage in the mPFC and hippocampus, leading to neurodegeneration.[39-43] The loss of neurons in this region disrupts the mPFC and critical hippocampal circuits, contributing to the memory deficits often seen in autism. In the mPFC and the CA2 region of the hippocampus, theaflavin resulted in an increased neuronal count, indicating a protective effect against VPA-induced neurotoxicity. Theaflavin's antioxidant properties likely play a role in reducing oxidative stress and neuronal damage in this critical region.

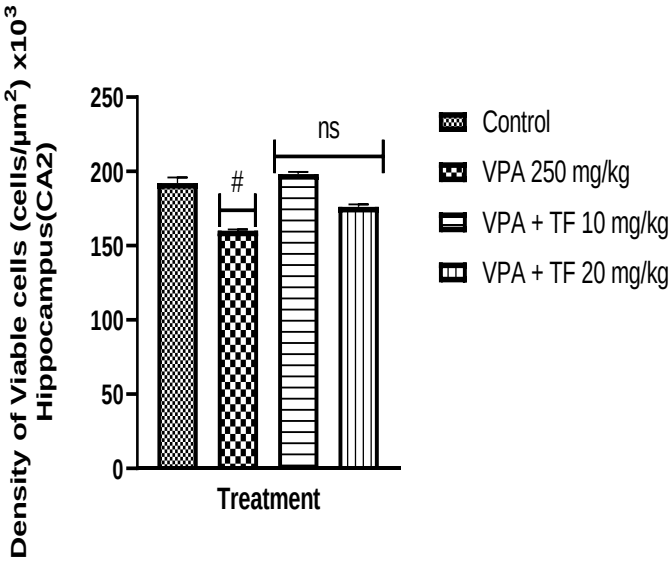

Figure 10b: The effect of theaflavin on the histology and neuronal density count of the cornu ammonis (CA2) of the hippocampi in prenatal valproic acid murine model.
Values represent the mean ± SEM for 5 animals per group
# $p<0.05$ compared to the control group (ANOVA followed by Tukey's posthoc test).
$p<0.05$ compared to the pathologic group (ANOVA followed by Tukey's posthoc test).
VPA: Valproic acid, TF: Theaflavin

Conclusion

This study suggests that theaflavin mitigates behavioural deficits in mice prenatally exposed to VPA by inhibiting oxidative stress and augmenting cholinergic activities. Hence, the findings from this present study indicate that theaflavin might be a potential treatment drug for the management of autism associated with oxidative stress and cholinergic alterations.

Conflict of Interests

The authors declare no conflict of interest.

Authors' Declaration

The authors of this article say that the ideas and work in it are their own and that they will be responsible for any claims that come up because of this article.

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