RESEARCH PAPER

Theaflavin and Anxiety Reduction in Zebrafish through GABAergic Modulation

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ABSTRACT

Anxiety issues are among the most common neuropsychiatric conditions, and safer, plant-derived anxiolytics are in increasing demand. Theaflavin, a polyphenolic compound found in black tea, possesses known neuroprotective properties and is also used in cardiovascular disease. This observation aimed to evaluate the anxiolytic potential of Theaflavin in zebrafish via behavioural and molecular checks, focusing on the GABAergic signalling pathway. Zebrafish had been divided into diazepam-treated and theaflavin-treated groups (1–one hundred µg/mL). Anxiety-like behavior became assessed using the light-darkish behaviors to take a look at (LDPT). Movement viability is evaluated using the MTT assay in KB cells to determine biocompatibility. For molecular evaluation, zebrafish brain tissues had been collected after behavioural testing, and qRT-PCR became useful to assess the expression of hysteria-related genes: gabra1, gabra2, gabrb1, gad1b, crh, and bdnf. Theaflavin-treated zebrafish exhibited more time within the light area in the LDPT, in particular at 50 µg/mL, indicating reduced anxiety-like behaviour akin to the diazepam institution. MTT assay showed that Theaflavin was non-poisonous as much as 50 µg/mL, with mild cytotoxicity found at 100 µg/mL. Gene expression evaluation showed significant upregulation of gabra1, gabra2, gabrb1, gad1b, and bdnf, along with downregulation of crh, most prominently at 50 µg/mL, helping the behavioural findings. Theaflavin shows anxiolytic results in zebrafish, probably mediated through modulation of the GABAergic system and pressure axis suppression. Those findings highlight Theaflavin as a promising, herbal anxiolytic candidate for similar neurobehavioral and healing research.

Key Words:

Theaflavin, Zebrafish, anxiety, GABAergic pathway, gabra1, crh, bdnf, light-darkish desire take a look at, MTT assay, Neuropharmacology, cardiovascular disease.

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INTRODUCTION

Tension issues are among the most common neuropsychiatric conditions globally, affecting over 264 million people and profoundly impacting life, overall cognitive performance, and emotional stability. **(1).** Characterised by means of worry, fear, and behavioural disturbances, tension arises from complex interactions between genetic, neurochemical, and environmental elements. Even though pharmacological interventions along with selective serotonin reuptake inhibitors (SSRIs), benzodiazepines, and serotonin-norepinephrine reuptake inhibitors (SNRIs) are mechanically prescribed, their lengthy-time period use is restricted through detrimental consequences, dependence potential, and delayed onset of action. This has generated a growing interest in plant-derived bioactives and dietary polyphenols that may provide anxiolytic results with minimal toxicity. Amongst such compounds, Theaflavin, a polyphenolic pigment formed during the oxidation of catechins in black tea (Camellia sinensis), has lately emerged as a promising candidate due to its neuroprotective, and antioxidant properties.

Zebrafish (Danio rerio) have proven to be a well-established vertebrate model for behavioural neuroscience and stress-related studies. The genetic and physiological homology of zebrafish to mammals, which includes similar neurotransmitter structures like GABAergic, dopaminergic, and serotonergic pathways, makes them an appealing model for preclinical screening of anxiolytic compounds. **(2)**

Zebrafish show quantifiable stress and anxiety-related behaviours, particularly in novel environments. Exams which include the mild-dark box check, Novel Tank Diving take a look at, and Social desire take a look at have been confirmed to assess tension-like responses. Particularly, the mild-darkish choice take a look at (LDPT) leverages the zebrafish's innate aversion to brightly lit environments to evaluate anxiety; improved time spent in the light quarter suggests reduced tension-like behaviour. On the neurochemical level, the γ-aminobutyric acid (GABA) machine plays a principal role in modulating anxiety. GABA is the number one inhibitory neurotransmitter within the central nervous system (CNS), and its binding to GABA_A receptors induces hyperpolarisation of neurons, resulting in anxiolytic effects. Dysregulation of GABAergic signalling has been implicated in anxiety problems, and many present-day anxiolytics, which include benzodiazepines, target GABA_A receptor subunits. In zebrafish and mammals alike, GABA_A receptor subunits including gabra1, gabra2, and gabrb1 encode the structural components essential for GABA-mediated inhibitory signalling. Moreover, gad1b, which encodes glutamic acid decarboxylase responsible for GABA synthesis, is an essential enzyme for preserving GABAergic tone within the brain. The interplay of those genes forms the molecular foundation of the GABAergic pathway's role in anxiety modulation.

Past the GABAergic device, neuroendocrine factors consisting of corticotropin-releasing hormone (CRH) and neurotrophic factors like brain-derived neurotrophic factor (BDNF) also make contributions to the stress phenotype. **(7)** CRH, released with the aid of the hypothalamus, initiates the hypothalamic-pituitary-interrenal (HPI) axis reaction to pressure in zebrafish, analogous to the hypothalamic-pituitary-adrenal (HPA) axis in mammals. Continual activation of CRH and the ensuing boom in cortisol levels can cause heightened anxiety-like behaviour. On the other hand, BDNF helps neuronal plasticity, resilience, and survival, and its dysregulation has been associated with tension and depression. Improving BDNF expression has shown beneficial consequences in assuaging tension in both preclinical and clinical research.

Theaflavin, abundant in black tea, is composed commonly of theaflavin-three,three'-digallate (TF3), theaflavin-3'-gallate, and theaflavin-three-gallate. Those polyphenols show off high antioxidant ability, chelate transition metals, and modulate several signalling pathways, including NF-κB, MAPK, and PI3K/Akt. Preceding research has tested the neuroprotective potential of theaflavins in ischemia models, Alzheimer's disorder pathology, and cognitive decline. **(3)**

But, the potential of Theaflavin in assuaging anxiety via modulation of GABAergic neurotransmission has no longer been very well elucidated, specifically in zebrafish models. Given the compound's acknowledged capacity to cross the blood-brain barrier and adjust oxidative and anti-inflammatory stress inside the CNS, investigating its position in behavioural and gene-level modulation of anxiety represents a unique and clinically relevant approach. To have a look at, we aimed to assess the anxiolytic ability of Theaflavin using a zebrafish model, integrating behavioural, mobile, and molecular techniques. The observed layout included the use of the MTT assay to determine the cytotoxicity of Theaflavin and make sure the concentrations used were biocompatible and non-toxic. Zebrafish larvae and adult models were exposed to Theaflavin at concentrations shown to be safe primarily based on MTT outcomes. Anxiety-like behaviour was assessed by the use of the mild-darkish choice check, quantifying the time spent in light versus dark compartments as an index of anxiety modulation.

In parallel, we investigated the expression of genes involved in GABAergic signalling (gabra1, gabra2, gabrb1, gad1b), the stress axis (crh), and neuroplasticity (bdnf) through qRT-PCR. This molecular analysis aimed to discover whether Theaflavin's anxiolytic effects had been associated with the upregulation of GABA receptor subunits and BDNF expression, in conjunction with the capacity downregulation of CRH, suggesting attenuation of HPI axis activation. The newness of this examination lies in the convergence of conduct-based screening and gene expression profiling to validate the anxiolytic capability of a naturally occurring, dietary polyphenol. If successful, this take-a-look-at should offer the idea for growing practical meals or phytopharmaceuticals incorporating Theaflavin for pressure and tension-related issues. Furthermore, the non-invasive and scalable zebrafish version used here can be tailored for high-throughput screening of other neuroactive nutraceuticals. In conclusion, the present observation explores the neurobehavioral and molecular results of Theaflavin on anxiety in zebrafish. Through focusing on the GABAergic pathway, CRH, and BDNF, we aim to explain the underlying mechanisms through which Theaflavin can also exert its calming impact. This painting contributes to the growing body of evidence supporting the therapeutic relevance of dietary polyphenols in neuropsychiatric situations and highlights zebrafish as a strong model for neurobehavioral pharmacology.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Theaflavin (> ninety-eight% purity) was received from Sigma-Aldrich (United States) and used for all experimental strategies. Stock solutions had been prepared in dimethyl sulfoxide (DMSO) and further diluted in system water to reach the favoured concentrations for in vivo exposure, with the final DMSO concentration maintained below 0.1% to keep away from solvent-associated toxicity. All reagents required for the MTT assay, which include the MTT reagent and dimethyl sulfoxide, in addition to RNA extraction reagents such as TRIzol and DNase, were also sourced from Sigma. For molecular evaluation, qRT-PCR reagents, such as cDNA synthesis kits and SYBR inexperienced master mix, together with specific primers focused on gabra1, gabra2, gabrb1, gad1b, crh, bdnf, and the house responsibilities gene β-actin, were procured from Bio-Rad.

2.2 Cellular tradition and situations

Human oral squamous carcinoma cells (KB mobile line) were used for in vitro cytotoxicity and molecular research regarding Theaflavin treatment. Cells were cultured in Dulbecco's modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, and maintained in a humidified incubator at 37 ± 1°C with a 5% CO_2 environment. The traditional medium was refreshed every forty eight hours to ensure the most beneficial mobile fitness. While cells reached eighty–ninety% confluence, they were subcultured using 0.25% trypsin-EDTA and reseeded at a density suitable for the unique assays. For MTT assays, KB cells were seeded at a density of one × 10^4 cells per well in 96-well plates and allowed to adhere for a single day before treatment. All experiments had been carried out using cells between passages 5 and 15 to preserve consistency in cellular conduct. Everyday commentary beneath an inverted microscope was achieved to monitor mobile morphology and exclude contamination. All approaches had been performed under strict aseptic conditions to ensure the reliability of experimental effects.

2.3 MTT Assay for Cytotoxicity Evaluation

The cytotoxic impact of Theaflavin on KB cells was evaluated using the usual MTT assay. KB cells have been seeded in 96-well plates at a density of 1 × 10^4 cells in line with the instructions and allowed to adhere for a single day in a humidified incubator at 37 ± 1°C with 5% CO_2. After 24 hours of attachment, the cells have been treated with Theaflavin at various concentrations: 1, 10, 25, 50, and one hundred µg/mL. A bad manipulated group (untreated cells) and an advantageous control organisation treated with Diazepam had been covered for evaluation. Each treatment was performed in triplicate. Following 24 hours of remedy, 20 µL of MTT reagent (5 mg/mL in PBS) was added to each well and incubated for 4 hours at 37°C. During this era, feasible cells transformed MTT into insoluble purple formazan crystals. After incubation, the media were cautiously removed, and a hundred µL of dimethyl sulfoxide (DMSO) was delivered to each properly to solubilise the formazan. Absorbance is measured at 570 nm with the use of a microplate reader. The share of mobile viability was calculated relative to the poorly managed group. Statistics were expressed as mean ± SD from triplicate wells. This assay helped decide the biocompatible concentration range of Theaflavin for subsequent behavioural and gene expression studies.

2.4 Zebrafish maintenance and ethical Approval

Wild-type zebrafish (Danio rerio) had been maintained in the institutional zebrafish facility under managed environmental conditions. Fish had been housed in 10-litre tanks with dechlorinated machine water, maintained at a temperature of 28 ± 1°C with a 14:10 hour mild-dark cycle. Water quality parameters, which include pH (7.zero–7.five), conductivity, and dissolved oxygen, were monitored regularly to ensure optimal conditions. Fish had been fed twice each day with an aggregate of business flake food and stay brine shrimp. Previous to experimentation, fish had been acclimatised to laboratory situations for not less than seven days. All approaches related to zebrafish were carried out according to the guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and were authorised via the Institutional Animal Ethics Committee (Approval No.: BRULAC/SDCH/SIMATS/IAEC/06-2023/15).

2.5 Evaluation of hysteria conducts the use of the light-dark cycle.

The light-darkish desire check (LDPT) was used to assess tension-associated conduct in adult zebrafish. A preferred glass tank measuring 20 cm × 10 cm × 10 cm was changed into a tank used for the assay, with the tank divided equally into booths: one transparent (light sector) and the other blanketed with black opaque paper (dark quarter) to create a contrasting environment. The water level is maintained at a level of 5 cm using filtered machine water. Zebrafish had been in my opinion added into the centre of the tank and allowed to freely explore both zones. Their movements have been recorded for a set duration using a top-mounted digital camera, and the time spent in each area was later analysed using tracking software. A desire for the mild area became interpreted as a reduction in tension-like conduct, reflecting the potential anxiolytic impact of the treatment administered.

2.5.1 light-darkish desire:

Experimental layout and manner

To evaluate tension-associated behaviour, the mild-darkish choice test (LDPT) was performed using adult

zebrafish. Fish have been randomly divided into 4 agencies (n = eight consistent with organisation): manipulate (untreated), DMSO control (0.1% DMSO), Theaflavin-treated (25 µg/mL), and Theaflavin-handled (50 µg/mL). The researchers take a look at changes carried out in a popular glass tank (20 cm × 10 cm × 10 cm), divided similarly into a light sector (transparent) and a darkish region (lined with black opaque paper) to simulate contrasting environments. The water stage was maintained at five cm using filtered machine water. Every fish changed into place for my part in the middle of the tank and allowed a 2-minute acclimatisation period, followed by a 5-minute rest period. Conduct became recorded by the usage of a digital digicam mounted above the tank, and the time spent in the mild zone was analysed using ANY-maze software program or ImageJ-based totally guide tracking. Accelerated time spent in the light area was interpreted as an anxiolytic-like impact, indicating a reduction in tension-like behaviour precipitated via Theaflavin treatment.

2.6 Gene Expression Analysis via qRT-PCR

To investigate the molecular mechanisms underlying the anxiolytic outcomes of Theaflavin, gene expression evaluation was completed using quantitative real-time PCR (qRT-PCR). Following the completion of behavioural assays, the zebrafish were euthanised, and the entire brains were cautiously dissected from 5 fish per group. The tissues had been pooled to achieve sufficient RNA yield and homogenised in TRIzol reagent according to the manufacturer's protocol. Overall RNA has been extracted, and its purity and concentration have been assessed using a NanoDrop spectrophotometer. One microgram of total RNA from each institution was reverse-transcribed into complementary DNA (cDNA) using the iScript cDNA Synthesis kit.qRT-PCR was performed using SYBR inexperienced master mix (Bio-Rad) on a Bio-Rad CFX96 real-Time PCR machine. Precise primers were used to make bigger genes related to the GABAergic system (gabra1, gabra2, gabrb1, gad1b), the stress axis (crh), and neurotrophic signalling (bdnf). The house responsibilities gene β-actin was used as an internal control to normalise gene expression levels. The PCR cycling situations blanketed an initial denaturation at ninety-five°C for three minutes, accompanied by forty cycles of denaturation at ninety-five°C for 15 seconds and annealing/extension at 60°C for 30 seconds. All reactions were finished in triplicate, and melt curve analysis was conducted to ensure specificity of amplification. Relative gene expression degrees have been calculated using the two^−ΔΔCt method, and effects have been expressed as fold changes compared to the managed institution. This evaluation enabled the evaluation of whether or not Theaflavin modulates key genes involved in anxiety regulation via GABAergic and neuroendocrine pathways.

3. RESULT

3.1 Morphological Observations of KB Cells

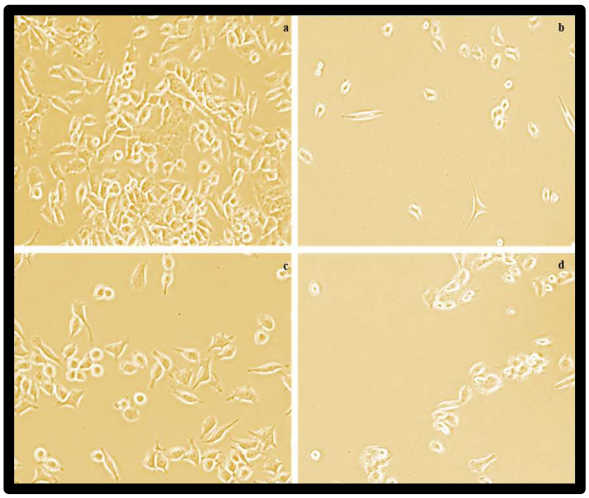

Figure 1: Consultant photos showing the morphological appearance of KB cells after treatment. Microscopic exam of KB cells following treatment with Theaflavin found dose-dependent morphological changes. Cells inside the negative control institution displayed wholesome, spindle-shaped morphology with uniform distribution and intact mobile integrity. The high-quality control (Diazepam) institution showed a slight reduction in mobile density however universally preserved mobile form. At lower concentrations of Theaflavin (1 and 10 µg/mL), cells seemed just like the untreated control, indicating excellent tolerance and minimum morphological disruption. However, at 25 µg/mL, moderate cell rounding and decreased confluence began to appear. Those modifications became more pronounced at 50 µg/mL, wherein cells exhibited shrinkage, partial detachment, and a less prepared monolayer. At 100 µg/mL, substantial morphological deterioration became obvious, with many cells dropping adherence, performing fragmentarily, and showing signs of cellular stress or apoptosis. These findings propose that Theaflavin is nicely tolerated at decreasing doses, even as higher concentrations may also compromise cellular integrity.

Figure 1: Consultant photos showing the morphological appearance of KB cells after treatment. a – terribly manipulate (untreated cells) displaying healthy, spindle-shaped morphology with an intact monolayer.

b – fine control (Diazepam-dealt with) showing mild discount in confluency.
c – Theaflavin-treated cells at 50 µg/mL showing mild shrinkage and decreased adherence.
d – Theaflavin-treated cells at 100 µg/mL showing great morphological disruption, detachment, and cell fragmentation.

3.2. Evaluation of Theaflavin-induced Cytotoxicity in KB Cells the usage of MTT Assay

The MTT assay effects for KB cells treated with various concentrations of Theaflavin revealed an attention-based response in cell viability. The poor control organisation exhibited continuously high absorbance values (218.01, 136.23, and 215. ninety four), indicating healthy and metabolically active cells without symptoms of cytotoxicity.

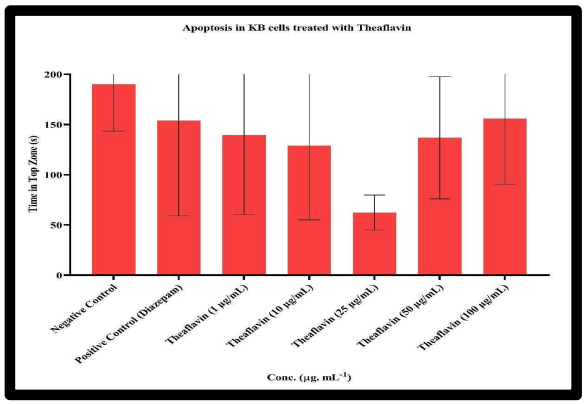

Figure 2: MTT assay showing the impact of Theaflavin on KB cell viability at concentrations of one, 10, 25, 50, and a hundred µg/mL, compared to the poor manipulation (untreated cells) and the superb control (Diazepam). Higher absorbance values suggest more cellular viability and metabolic activity.

In contrast, the positive management organisation treated with diazepam confirmed variable absorbance (54.42, 242.97, and 163.93), suggesting inconsistent cell response, however an unusual reduction in viability compared to the negative control. At low concentrations, Theaflavin (1 and 10 µg/mL) verified minimal cytotoxicity, with high absorbance values in most replicates—especially at 1 µg/mL (forty eight.31, 188.09, and 182.19) and 10 µg/mL (211.sixty eight, 106.39, and sixty nine.02)—indicating accurate tolerance and sustained metabolic activity. However, at 25 µg/mL, a substantial decline in absorbance was found (79.three, 44. forty eight, and sixty three.17), pointing to reduced viability and increased cytotoxic effects. remedy with 50 µg/mL Theaflavin confirmed a combined sample (195.99, seventy four.39, and 139. ninety one), suggesting moderate cytotoxicity with some cellular populations remaining possible. At 100 µg/mL, the absorbance values (a hundred and sixty.2, 218. ninety four, and 88.2) were enormously variable, reflecting inconsistent cell responses, even though usually lower than the control, indicating that higher doses may impair cell viability. The results recommend that Theaflavin is biocompatible at lower concentrations (1–10 µg/mL), even as higher concentrations (25 µg/mL and above) may additionally induce dose-dependent cytotoxic outcomes in KB cells.

3.3 Anxiolytic outcomes of Theaflavin in Zebrafish Assessed with the aid of mild-darkish choice behaviour

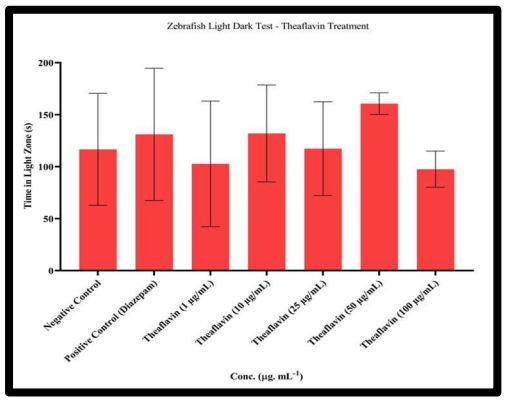

Figure 3: mild-dark desire check showing the time spent within the light region by way of zebrafish after treatment with Theaflavin at concentrations of 1, 10, 25, 50, and 100 µg/mL, in comparison to the poor control (untreated) and superb control (Diazepam-treated). Increased time in the light quarter indicates decreased tension-like conduct and potential anxiolytic results.

The results from the light-darkish choice test (LDPT) monitor wonderful behavioural responses in zebrafish following Theaflavin treatment at varying concentrations. The poor managed institution exhibited moderate time spent within the light sector (178.69, 82. seventy two, and 88. fifty two), reflecting baseline tension-like conduct ordinary in untreated zebrafish, which certainly prefer the darker area due to their scototactic nature. The fine-managed organisation treated with Diazepam, a recognised anxiolytic, showed improved time in the mild area (61.77, 186. fifty five, and 144.91), indicating reduced anxiety and validating the test's sensitivity. Zebrafish treated with Theaflavin at 1 µg/mL showed variable responses (50, 89. four, and 168.65), suggesting a moderate anxiolytic impact in a few people. At 10 µg/mL, the values (147.eight, 168.54, and seventy nine.48) indicate a greater steady increase in time

spent within the mild sector, suggesting better anxiolytic-like behaviour. remedy with 25 µg/mL Theaflavin (159.52, 69. eight, and 122.58) also verified increased mild area desire in most replicates, in addition to supporting a dose-responsive anxiolytic impact. Extensively, 50 µg/mL Theaflavin confirmed the best consistency and typical growth in time spent within the light area (152.65, 156. eight, and 172. forty two), suggesting this as the only dose for tension reduction. However, at one hundred µg/mL, time inside the light quarter reduced barely (85.76, 117. fifty two, and 89.38), indicating potential loss of efficacy or onset of strain at higher concentrations. Typically, Theaflavin tested a dose-dependent anxiolytic effect in zebrafish, with 10–50 µg/mL displaying the maximum steady development in light quarter choice, indicative of reduced tension-like behaviour.

3.4 Modulation of hysteria-associated Genes via Theaflavin in zebrafish brain

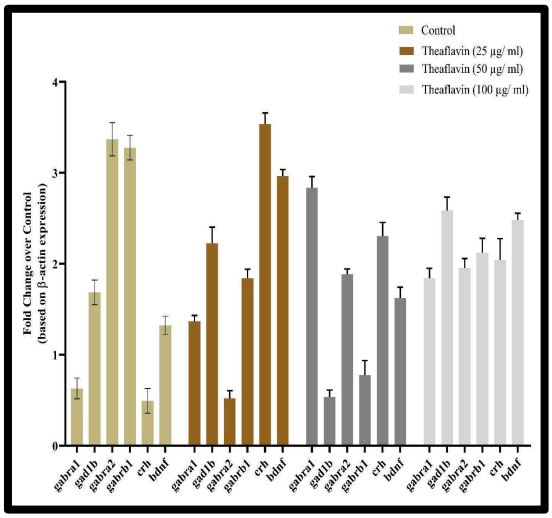

Figure 4: Relative gene expression degrees of tension-associated markers (gabra1, gabra2, gabrb1, gad1b, crh, and bdnf) in zebrafish mind following Theaflavin treatment at 25, 50, and one hundred µg/mL. Expression levels have been normalised to β-actin and offered as fold adjustments in comparison to the untreated control. Upregulation of GABAergic and neurotrophic genes with concurrent downregulation of CRH indicates anxiolytic capacity, most prominently at 50 µg/mL.

Gene expression analysis was carried out to assess the molecular effect of Theaflavin on tension-related pathways in zebrafish brains. The observation targeted the GABAergic signalling genes (gabra1, gabra2, gabrb1, gad1b), stress reaction gene (crh), and neurotrophic marker (bdnf) at three Theaflavin concentrations: 25, 50, and one hundred µg/mL. At 25 µg/mL, there was a moderate upregulation of gabra1 and gad1b, suggesting improved GABA synthesis and receptor activation, indicative of a mild anxiolytic response. gabrb1 and gabra2 showed a mild increase, at the same time as bdnf became mildly multiplied, implying neurotrophic help. CRH expression became barely downregulated, reflecting decreased strain-axis activation. At 50 µg/mL, the maximum stated effects have been located. massive upregulation of gabra1, gabra2, gabrb1, and gad1b indicated a strong activation of the GABAergic inhibitory pathway. BDNF tiers had been additionally strongly increased, correlating with more advantageous neuroplasticity and emotional law. Simultaneously, crh expression became significantly downregulated, indicating suppression of the HPI (hypothalamic-pituitary-interrenal) pressure axis, consistent with behavioural findings from the LDPT. At one hundred µg/mL, the gene expression profile started to change barely. Even though gabra1 and gad1b remained upregulated, their degrees have decreased to 50 µg/mL. Gabra2 and Gabrb1 confirmed minimal adjustments, and bdnf expression plateaued or declined slightly. Considerably, crh expression began to upward thrust once more, suggesting a possible pressure reaction or toxicity at this higher awareness. Theaflavin treatment modulated key genes involved in anxiety regulation, with 50 µg/mL rising as the best dose for boosting GABAergic signalling, boosting BDNF expression, and suppressing strain markers, supporting its anxiolytic potential in zebrafish.

4. Discussion

Tension issues, marked via excessive fear and behavioural disturbances, are a global mental fitness challenge, with excessive prevalence and great socio-economic effect. **(10)** While modern pharmacotherapies including benzodiazepines and SSRIs are effective for lots of sufferers, they're regularly related to unfavourable effects like sedation, dependency, and tolerance, mainly to a developing interest in natural, plant-derived options. This examination aimed to research the anxiolytic potential of Theaflavin, a polyphenolic compound from black tea, in zebrafish using behavioural evaluation and molecular profiling, with a focus on the GABAergic pathway and stress-related gene law. The mild-darkish desire check (LDPT) became the number one behavioural assay for tension. Zebrafish, because of their innate scototaxis (preference for dark areas), certainly keep away from properly lit zones.**(4)** However, anxiolytic compounds lessen this avoidance, leading to accelerated time spent inside the light zone. Within the gift look at, zebrafish exposed to Theaflavin exhibited a dose-structured increase in time spent inside the mild zone, with 50 µg/mL generating the most regular and sizable behavioural

change. Those findings mirror the anxiolytic impact discovered within the diazepam-treated institution and suggest that Theaflavin at intermediate concentrations can successfully lessen tension-like behaviour. Lower concentrations (1 and 10 µg/mL) confirmed variable effects, probably because of insufficient systemic availability or subthreshold neurochemical outcomes, even as a hundred µg/mL verified a decline in behavioural performance, possibly due to strain or mild toxicity, as supported by way of each behavioral and gene expression profiles.

To ensure the safety of Theaflavin at the examined doses, an MTT assay was carried out using KB cells. The outcomes showed that Theaflavin is non-poisonous at up to 50 µg/mL, with some degree of metabolic suppression determined at 100 µg/mL. **(5)** Morphological observations supported those findings, with normal spindle-shaped cells at lower doses and evidence of shrinkage, detachment, and rounding at higher concentrations. This cytotoxic profile reinforces the behavioural findings, indicating 50 µg/mL as a biologically energetic yet biocompatible dose suitable for neurobehavioral studies. At the molecular level, gene expression profiling supplied insight into the mechanistic foundation of Theaflavin's anxiolytic activity. The GABAergic system is central to tension regulation, because it exerts inhibitory manipulation over excitatory neurotransmission. especially, GABA_A receptor subunits (encoded by means of gabra1, gabra2, gabrb1) and the enzyme gad1b, which synthesises GABA from glutamate, are essential in keeping inhibitory tone inside the brain. Theaflavin remedy caused a dose-structured upregulation of those genes, most importantly at 50 µg/mL. This upregulation possibly reflects improved GABA synthesis and receptor responsiveness, contributing to decreased neuronal excitability and anxiety suppression. The growth in gad1b additionally shows a shift towards better GABA availability, further assisting an anxiolytic environment within the zebrafish brain.

Similarly to the GABAergic pathway, the neuroendocrine strain reaction was evaluated through CRH (corticotropin-releasing hormone) expression. CRH is a key regulator of the hypothalamic-pituitary-interrenal (HPI) axis in zebrafish and has similarities to the HPA axis in mammals. Accelerated CRH is related to stress and tension, mainly to cortisol release and behavioural disruption. On this look at, Theaflavin downregulated crh expression, specifically at 50 µg/mL, indicating suppression of the pressure axis. This suggests that Theaflavin now not only enhances inhibitory neurotransmission but also reduces neuroendocrine strain signalling—two crucial components of a comprehensive anxiolytic impact. Another key finding became the upregulation of BDNF (brain-derived neurotrophic factor), a gene crucial for synaptic plasticity, neuronal survival, and emotional law. **(8)** BDNF is frequently downregulated below continual strain and anxiety conditions. Theaflavin remedy reversed this fashion, mainly at 50 µg/mL, where a marked boost in bdnf expression was observed. This supports the idea that Theaflavin no longer best mitigates tension but may additionally sell neuronal resilience and healing. The twin upregulation of BDNF and GABAergic genes shows a synergistic impact, improving both synaptic inhibition and neural plasticity, which may additionally contribute to long-time period behavioral advantages. Interestingly, the one hundred µg/mL institution confirmed a relative decline in the expression of key genes together with gabra2, gabrb1, and bdnf, together with a partial rebound in crh expression. This fashion indicates a capability dose threshold beyond which Theaflavin can also elicit pressure or poisonous responses, blunting its anxiolytic capacity. The behavioural information from the LDPT corroborates this, as zebrafish in the one hundred µg/mL group spent much less time in the light sector as compared to the ones treated with 50 µg/mL. Together, these outcomes underline the importance of figuring out prime healing windows for natural compounds, as higher doses do not usually translate to more efficacy.

The zebrafish model used in this observation affords numerous benefits for neurobehavioral pharmacology. **(9)** Its conserved neurotransmitter structures, obvious development, and high-throughput capability make it a great organism for early-stage drug screening. Furthermore, the reproducibility of the LDPT and the feasibility of brain dissection for gene expression analysis strengthen the model's translational relevance. This observation aligns with different findings demonstrating the utility of zebrafish for evaluating herbal anxiolytics, together with those derived from flavonoids, alkaloids, and polyphenols. The modern findings surround Theaflavin amongst other promising polyphenols with neurobehavioral advantages. Previous research has said that Theaflavin can reduce neuroinflammation, oxidative stress, and neuronal apoptosis in models of Alzheimer's disease and stroke. **(3)**

Its potential to move the blood-mind barrier and modulate imperative signalling pathways such as PI3K/Akt and NF-κB in addition to its neurological relevance. However, limited research has explored its impact on emotional behaviour, specifically tension, making this research a treasured addition to the literature.

No matter the promising outcomes, this take a look at has a few obstacles. First off, while the LDPT is a tested assay for tension-like conduct, additional exams consisting of the novel Tank Diving take a look at or

Social choice test could have bolstered behavioural interpretations. Secondly, cortisol levels were not measured immediately, which could have in addition shown pressure-axis involvement. Thirdly, protein-stage validation of gene expression changes (e.g., through Western blot or immunostaining) was not accomplished, leaving the useful translation of transcriptional modifications open to investigation. Lastly, they have a look at becoming restrained to acute exposure; chronic exposure models would help evaluate the long-time period safety and efficacy of Theaflavin.

Future studies should recognise combining behavioural, biochemical, and electrophysiological techniques to fully elucidate the anxiolytic mechanism of Theaflavin. Research using anxiety-inducing paradigms (e.g., caffeine-triggered or chronic pressure paradigms) might provide insights into its therapeutic capability in ailment-like situations. Investigating Theaflavin's interplay with other neurotransmitter systems, along with serotonin or dopamine, might also increase information about its neuropharmacological profile. Moreover, comparative studies with different tea polyphenols, together with EGCG or catechin, could help identify synergistic or superior compounds for intellectual fitness programs. In the end, this take a look demonstrates that Theaflavin exerts significant anxiolytic consequences in zebrafish through behavioural improvement within the light-dark preference test and modulation of genes associated with GABAergic signalling, neurotrophic support, and stress regulation. The findings assist Theaflavin as a promising herbal candidate for anxiety management and lay the foundation for future translational studies involving plant-based therapeutics for mental health. **(6)**

This provides compelling evidence that Theaflavin, a bioactive polyphenol from black tea, exhibits tremendous anxiolytic consequences in zebrafish. Behavioural evaluation via the mild-darkish choice check discovered a dose-based reduction in tension-like behaviour, with 50 µg/mL identified as the best concentration. Complementary gene expression research verified upregulation of key GABAergic markers (gabra1, gabra2, gabrb1, gad1b) and the neurotrophic aspect bdnf, along with downregulation of the strain-related gene crh, suggesting that Theaflavin modulates both inhibitory neurotransmission and strain-response pathways. Additionally, MTT assay results confirmed that Theaflavin is biocompatible at up to 50 µg/mL, with minimal cytotoxicity observed. Collectively, these findings spotlight Theaflavin as a promising natural anxiolytic agent that exerts its effects through GABAergic and neurotrophic mechanisms and highlight its potential for development in neurobehavioral therapeutics. Further research involving chronic exposure models and protein degree validations is endorsed to fully elucidate its therapeutic profile.

CONFLICT OF INTEREST

There is no conflict of interest.

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