

<https://doi.org/10.48047/AFJBS.7.10.2025.248-264>



African Journal of Biological Sciences

Journal homepage: <http://www.afjbs.com>



Research Paper

Open Access

## Exploring the Therapeutic Potential of Probiotics: A Zebrafish Model for Stress Management

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Volume 7, Issue 10, 2025

Received: 14 July 2025

Accepted: 25 Sep 2025

Published: 13 Oct 2025

Doi :[10.48047/AFJBS.7.10.2025.248-264](https://doi.org/10.48047/AFJBS.7.10.2025.248-264)

### Abstract

Stress management in zebrafish is a significant area of research, and probiotics may aid in alleviating stress. Probiotics, which are live bacteria that can make you healthier if you take enough of them, have largely been studied in the gut microbiota and immune systems of animals. But more and more people are using them on zebrafish, which have a very good way of dealing with stress. Zebrafish are excellent models for investigating stress-induced behaviours and physiological alterations resulting from overcrowding, temperature fluctuations, and chemical exposure. Probiotics may regulate stress-related pathways and neurotransmitter systems in zebrafish, mitigating behavioural and physiological symptoms associated with stress. The main results suggest that probiotics can change the makeup of the gut microbiota and improve intestinal health, which can help with stress-related reactions. The impact of probiotics on zebrafish physiology and behaviour elucidates stress-related disorders and presents potential therapies for humans and other organisms. This abstract examines research in this emerging field and underscores the necessity of comprehending the intricate interplay of probiotics, stress, and the welfare of aquatic organisms.

### Keywords

Behaviour analysis, Early life stress, Gut dysbiosis, Gut brain axis, Probiotics, Stress management, Zebrafish.

## **Introduction**

Chronic stress is a major contributor to poor health, which is associated with a broad range of problems such as reduced productivity, metabolic syndrome, mental health disorders, cardiovascular diseases and thus leading to increased healthcare costs. To combat this, interactive technologies and stress-reduction techniques must be widely deployed and applied (Russell et al, 2019 & Nicolas Rohleder, 2019). Chronic mood disorders like anxiety and depression often arise from unexpected stress sources, and it is associated to numerous human illnesses. Purinergic signaling interacts with other signal molecules to form a complex network and thus regulate physiological processes such as cellular proliferation, differentiation and death. Purines, most notably adenosine and ATP are the key molecules that control the intracellular energy homeostasis and nucleotide synthesis (Huang et al, 2021). Early-life stress (ELS) can have long-lasting impacts on behavior in adults, including enhanced resilience and susceptibility to illness (Fontana et al, 2021). Despite early life vulnerability to psychiatric disorders, locomotion and exploratory activity are the best stress predictors. Exploratory factor analysis suggests early-life stress weakens the arousal-inhibition system that leads to prolonged reactions and slower weight development (Sequeira-Cordero et al, 2019). Unhealthy lifestyles contribute most in the premature mortality of humans and lifestyle medicine focuses on health risk behaviors and self-management. It includes diet, physical activity, behavior change, weight control, stress, coping, and spirituality and mind-body techniques (Friedman & Susan, 2020). Diet, obesity, metabolic syndrome, stress, and mental illnesses are interconnected. Dietary changes and genetic susceptibility can influence stress-related mental illnesses like obesity, depression, and PTSD. So, understanding the stomach-brain connection could improve treatment for stress and obesity-related mental health issues (Bremner et al, 2020).

Stress can disrupt homeostasis and affect the digestive system which leads to disorders like Functional Gastro Intestinal Disorders (FGID), Irritable Bowel Syndrome (IBD) and adverse reactions. Glucocorticoid hormones are released in response to stress which leads to a range of biological changes such as immune modulation and energy

metabolism (Labanski et al, 2019). Immunological pathway is another way of modulating the microbiota by stress with the help of proinflammatory cytokines. The psychological stress affects intestinal sensitivity, motility, secretion, and permeability and leads to immunological activation which causes changes in the central nervous system, peripheral neurons, and gastrointestinal flora (Molina-Torres et al, 2019). The pathophysiology of mental illnesses may be influenced by disruptions in metabolic pathways and the hypothalamic-pituitary-adrenal axis. Intestinal metabolites such as short-chain fatty acids and bacterial components can boost the immune system (Sabit et al, 2023). Gut bacteria can cause pathogenic diseases including neurological and psychiatric disorders. Gut dysbiosis is associated with neurodegenerative conditions like Alzheimer's, Parkinson's, Huntington's, motor neuron disease, and multiple sclerosis (Gubert et al, 2020). In addition, alterations in the gut microbiota-brain axis are related to cognitive abilities. Spinal cord injuries can cause anxiety and depression leading to loss of independence and psychological pressures. Brain regions involved in emotion and information processing may be affected by mood and cognition changes (Musleh-Vega et al, 2022). Agitation, despair, apathy, compulsive questioning, psychosis, and violence are some of the behavioral and psychological signs of cognitive disorders (Jing et al, 2020). Research in nutritional psychiatry suggests that healthy eating habits can prevent and treat depression symptoms through various pathways including oxidative stress, epigenetic, mitochondrial dysfunction, gut microbiota, tryptophan-kynurenine metabolism, HPA axis, neurogenesis, and obesity (Marx et al, 2021).

Probiotics are being used to improve health and well-being, with evidence suggesting direct and indirect interactions between gut microbiota and the central nervous system. The improvement in GM diversity reduces anxiety and depressive-like behaviors (Liu et al, 2020). Probiotics and Prebiotics have been found to improve cognitive function and reduce the prevalence of mental diseases like anxiety, depression, and autism. The development of "psychobiotic" medications aims to provide anti-inflammatory, antidepressant, and anti-anxiety benefits with minimal side effects (Ansari et al, 2020). Probiotic administration significantly reduces depressive symptoms with the most significant factors being the probiotic composition, the quantity of ingested psychobiotics, and the duration (Zagórska et al, 2020). Gut microbiota bacteria composition can affect physical activity, and probiotics can improve exercise tolerance and physical capability (Marttinen et al, 2020).

Zebra fish is a vertebrate closely related to our species which has been a significant player in developmental biology, toxicology, and pharmacology research and is currently gaining attention in neurology. This Zebra fish model is a versatile tool for behavioral brain study and ideal for large- scale screening experiments to find new drugs or mutants with altered characteristics. It's also suitable for studying biological learning and memory pathways, as it can be used for mutagenesis screening (Trigueiro et al, 2020). Zebra fish, unlike rat models, can induce behavioral, physiological, and molecular changes similar to those in depressed individuals. Researchers studied zebra fish's response to unexpected chronic stress (UCS) and (Marcon et al, 2018) revealed UCS causes higher anxiety and promotes the expression of pro-inflammatory cytokine genes like IL-1 $\beta$  and TNF- $\alpha$ . This method is faster than traditional mouse models and supports neurobiology investigations, making it suitable for preclinical stress investigations (Karina et al, 2021& Marcon et al, 2018). One of the studies used zebra fish (*Danio rerio*) as a model to investigate the neurobehavioral effects of unpredictable chronic stress. Alteration of brain proteome profile was found which implicated the mitochondrial dysfunction of chronically stressed zebra fish (Konstantin et al, 2021). Environmental enrichment promotes neuroprotection and neurogenesis, reducing stress susceptibility. Zebra fish model suitable to studies EE's behavioral effects, while exercise mitigates stress and enhance overall well-being and reduce vulnerability (Marcon et al, 2018).

## **Materials and Methods**

### **Experimental groupings**

The zebrafish experiments were conducted in compliance with ethical approval guidelines set forth by CCSEA (MB/IAEC/2023/01/12). Zebra fish used in this study were procured from Satybama Institute of Science and technology, Chennai. The fishes were divided into three groups which were housed in 30 L tanks and the temperature of the tanks was maintained at  $25 \pm 2^\circ\text{C}$ , and the photoperiod was maintained 12/12 h light/dark cycle. The experimental protocols were approved by Institutional Ethical Committee. Control (non-stressed) group (Group I) fishes were kept in a controlled environment without any external stressors. They were maintained under standard temperature and conditions throughout the entire duration of the experiment. This group served as the baseline against which the other two groups would be compared. Stress induced fishes (group II) were exposed to heating tank water to  $33^\circ\text{C}$  for 30 minutes at particular times (8:00 am and 5:00 pm) daily for 14 days. This thermal stress was

maintained consistently over a period of 14 days, simulating a challenging environmental condition that aquatic organisms might encounter in the wild. Treatment group (Group III) were induced stress by allowing it in heating tank water to 33°C for 30 minutes at particular times (8:00 am and 5:00 pm) daily for 14 days similar to the same thermal stress conditions as Group II and this group received probiotics “*Bacillus coagulans*” (500 µg/kg body weight). This treatment was administered to explore the potential mitigating effects of probiotics on the stress-induced responses of the fish.

### **T- Maze analysis of control and experimental grouping**

T-Maze analysis is a research method used in neuroscience and behavioral studies to study learning, memory, and decision-making in zebra fish. These fishes are popular due to their genetic tractability and ability to manipulate their neural circuits. The TMaze apparatus consists of a transparent tank with a start arm and two horizontal arms as choice arms. During the T-Maze analysis, all groups of zebra fish were subjected to the same training and testing protocols, ensuring consistency in the experimental conditions. During testing, zebra fish from three groups were placed in the start arm and allowed to make choices. Observations are recorded and data from control and experimental groups are compared to assess differences in learning and memory performance. Neurobiological correlates of T-Maze performance are found in specific brain regions and neural pathways. Repeated trials are conducted to ensure consistent choices over time (Ngoc et al, 2020).

### **Biochemical parameters Analysis of Superoxide dismutase (SOD)**

Initially, 0.1 ml of tissue homogenate is used as a source of the enzyme under investigation. To this 0.1 ml of tissue homogenate, 0.5 ml of EDTA solution and 0.5 ml of carbonate buffer are added. The carbonate buffer provides a suitable pH environment for the enzyme to function optimally. The total volume of the reaction mixture is adjusted to 2.5 ml, possibly by adding a suitable solvent or buffer to reach the desired volume. The enzymatic reaction is initiated by adding 0.4 ml of epinephrine to the mixture. The increase in absorbance at 480 nm is monitored over time using a UV spectrophotometer. 50% of auto oxidation of epinephrine to adrenochrome was performed in a control tube without the enzyme. It serves as a baseline for comparison to determine the specific contribution of the enzyme to the reaction. The enzyme activity was expressed as IU/mg of protein.

**Catalase (CAT) assay**

The process begins by homogenizing the tissue using an isotonic buffer and breaks down the tissue into smaller fragments and disrupts cell membranes. Then homogenized sample is subjected to centrifugation at 3000 rpm for 10 minutes at a low temperature of 4°C. The pellet, which contains cellular debris and potentially unbroken cells are discarded and leaving behind the clear supernatant which contains soluble components including catalase. To the supernatant, 0.01 ml of ethanol is added per ml of supernatant liquid. The samples are then incubated in an ice water bath for 30 minutes. Following incubation, 10% Triton X-100 is added to 0.1 ml of the supernatant and used to enhance the accessibility of catalase for the subsequent catalytic reaction. For the catalase estimation, 2 ml of the sample is diluted in phosphate buffer. To initiate the reaction, 1 ml of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is added to the mixture and the rate of decomposition of H<sub>2</sub>O<sub>2</sub> is measured by monitoring the change in absorbance at 240 nm. This measurement is taken at 15-second intervals for a total of 3 minutes. The catalase activity is expressed as μmoles of H<sub>2</sub>O<sub>2</sub> decomposed / min/ mg protein.

**Estimation of Glutathione peroxidase (GPx)**

Glutathione peroxidase activity was assayed by the method of Rotruck et al., (1973) with slight modifications. An aliquot of sample was taken with 0.4ml of sodium phosphate buffer and 0.1ml of sodium azide. Sodium phosphate buffer helps maintain a stable pH environment for the enzymatic reaction, and sodium azide inhibits microbial growth or unwanted reactions. To this, 0.2ml of reduced glutathione and 0.1ml of H<sub>2</sub>O<sub>2</sub> was added and the total volume of the reaction mixture is made up to 2.0 ml by adding distilled water. Then, the reaction mixture was incubated for 3min at 37 °C and the reaction was stopped by adding 0.5ml of 10 %TCA. After this, the contents were centrifuged and the supernatant was removed and pellet was taken and to this 4.0ml of disodium hydrogen phosphate and 1ml of DTNB was added and read at 412nm using Shimadzu UV spectrophotometer against reagent blank. Glutathione peroxidase activity is expressed as nmoles of GSH oxidized/min/mg of protein.

**Assay of Lipid peroxidation (LOOH)**

The Lipid peroxidation levels of the protein samples of three groups were estimated using Fox's Reagent. The Fox's reagent is prepared by adding 100μl Xylenol orange, 11 mM Butylated hydroxyl toluene, 25 mM Sulphuric acid, 25 μM Ferrous Sulphate, 90 ml methanol, and 10 ml of 250 mM Sulphuric acid. A total of 50 μL of the protein samples from each of the three groups is used for the assay. To each 50 μL protein

sample, 0.9 ml of Fox's Reagent is added. The mixture of protein sample and Fox's Reagent is incubated at room temperature for 30 minutes. After the 30-minute incubation, the absorbance of the samples is measured at a specific wavelength of 560 nm using a UV spectrophotometer with a reagent blank. The amount of hydro peroxides produced was calculated by using a molar extinction coefficient of  $4.6 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ . The levels of LOOH are expressed as nmoles/g tissue.

### **Quantitative real time PCR analysis of Stress marker genes of control and Experimental group fishes**

Total RNA from the liver tissue was isolated and quantified according to Chomczynski & Sacchi, 1987 method. Reverse transcription (RT) was used to derive the cDNA from the total RNA using a cDNA conversion kit from Thermo Scientific Inc. Then 1.5 l of random hexamer primer was added to the reaction mixture, which included 0.5-1.0 g of total RNA, and incubated at 72 °C for 10 minutes before being promptly refrigerated. To this, 5.0µL of premixed 10 mmol/L deoxy nucleotide triphosphate (dNTP) solution, 3.0µL 10x Moloney Murine leukemia virus (M-MLV) RT buffer, and 1.0µL of M-MLV RT was added and made up to 50µL using RNase/DNase free water. Further, the real time PCR was performed on BioRad CFX96 with SYBR green fluorescent label. GAPDH was used as an internal control.

### **Statistical analysis**

Data obtained in the study were expressed as mean  $\pm$  S.D (n=6). Statistical analysis was carried out using SPSS software, version 16.0 with a student t-test. The data shown are presented with mean  $\pm$  SEM with p value less than 0.05 regarded as statistically significant.

## **Results and Discussion**

### **T-maze analysis and Experimental Grouping**

As represented in Figure 1, the T-Maze analysis in a zebra fish model reveals that the treatment group of zebra fishes showed improvements in choice behavior and decisionmaking strategies than stress induced fishes. This finding suggests that treatment group may adopt more efficient strategies which lead to quicker and more accurate choices than induced group. As probiotic treatment improved cognitive function, it might exhibit shorter decision-making times, potentially leading to quicker learning and fewer incorrect choices.

### **Histology**

The control and experimental brain tissues of stress induced zebra fishes showed degeneration of purkinje layer and sever vacuolation when compared to control. Fish from treatment group showed a lesser vacuolation in the tissue but also, purkinje cells appeared to be non-visible even in the treated group as shown in Figure 2.

### **Biochemical assays:**

The biochemical analysis of control and experimental tissues were represented in Figure 3. Protein carbonylation is a result of oxidative damage to proteins, where reactive oxygen species (ROS) or reactive nitrogen species (RNS) modify amino acid residues, particularly lysine, arginine, proline, and threonine, resulting in the formation of carbonyl groups. Measurement of protein carbonyl content can indicate the level of protein oxidation and serve as an indirect marker of oxidative stress. Protein carbonyl content is higher in stress induced group compared to control group, while probiotics treated group lowered the level of protein oxidation slightly. The potential impact of probiotics on the oxidative stress and antioxidant defense systems in zebra fishes can be assessed by the levels of antioxidant enzymes such as Superoxide dismutase - SOD, Catalase - CAT, Glutathione peroxidase - GPx and lipid peroxidation (LOOH). Lipid peroxidation is the process of oxidative degradation of lipids, particularly polyunsaturated fatty acids, by free radicals or other reactive species. This process generates lipid peroxides, which can further react and cause damage to cellular membranes and other lipids. Lipid peroxidation is often assessed by measuring the levels of malondialdehyde (MDA) or other similar byproducts, which are indicators of lipid peroxidation and oxidative stress. Stress-induced group had a higher level of LOOH than control while treatment group slightly reduced the level of LOOH. Superoxide Dismutase (SOD) is an enzyme living in all living organisms and it helps to break down potentially harmful oxygen molecules in cells. Stress induced group showed a reduction in the level of SOD than control. Treated group showed a significant increase in the enzyme levels indicating the positive impact of probiotics. Glutathione Peroxidase (GPx) is one of the enzymes in the body to protect the organism from the oxidative damage. Stress induced group of zebra fishes showed tremendous decrease in the enzyme activity when compared to control while probiotics treated group had a significant incline in the level of GPx indicating potential use of probiotics treating organisms with chronic stress.

**Gene expressional analysis of control and experimental grouping Stress markers:**

GABA (Gamma-aminobutyric acid) is the principal inhibitory neurotransmitter in the CNS and is opposed by the excitatory neurotransmitter glutamate. A disruption in their balance results in CNS malfunction (Figure 4). There are two receptors for GABA; GABA A and GABA B. Activation of GABA-A cause hyper polarization to decrease the likelihood of an action potential while GABA B involves in the modulation of intracellular activity and neurotransmitter release. Expression of both receptors tends to be low in normal conditions as seen in control. Stress induced group of zebra fish expressed a high level of both GABA A and GABA B receptors while the treatment group showed a significant reduction which suggest a potential reduction in stress and anxiety, indicating a calming effect of probiotics.

Corticotropin Releasing Factor (CRF) is one of the major hormones and at the same time nucleopeptide acting during stress response. CRF is up-regulated in the stressinduced group of zebra fishes than the control group under normal and controlled conditions. It is down regulated in the treatment group where the stressed fishes were treated with probiotics indicating the positive impact of probiotics during stress. Calcineurin, a calcium-calmodulin-activated phosphatase consisting of a heterodimer with the catalytic and regulatory subunits and controls the expression of several genes by dephosphorylating the transcriptional regulator Crz1 through binding to calmodulin, a calcium sensor. It plays many important physiological roles which also include learning and memory, and it is 10-20 times more concentrated in brain than in other organs. Up-regulated levels of Calcineurin occurred due to stress induction is seen down-regulated in the treatment group of zebra fishes. This indicates a positive effect of probiotics.

PHB2 is a conserved IMM protein that functions in diverse cellular processes including mitochondrial dynamics. Stress induced group showed increased levels of PHB2 than control which is down regulated notably in probiotics treated group of zebra fishes. Brain Derived Neurotrophic Factor (BDNF) is a key molecule involved in the changes related to learning and memory. The levels of BDNF showed an increase in stress induced zebra fishes while it decreased slightly after the treatment with probiotics. Stress is a major contributor to anxiety disorders and several studies evident that zebra fish is suitable for behavior analysis and neurological studies. Zebra fish larvae can be used to study early life stress and its impact on adulthood behavioral characteristics, including learning and anxiety (Fontana et al, 2021). In the similar study, zebra fish are

experiencing physiological stress and sensory impairment due to anthropogenic noise in aquatic habitats. Behavior analysis showed all fish spent more time on the bottom during the first minute, indicating anxiety or stress (Wong et al, 2022). Zebra fish anxiety models were used to understand the N-Acetylcysteine amide (NAC) effect on behavioral and physiological markers (Reis et al, 2020). The unpredictable chronic stress (UCS) in zebra fish found that it increased anxiety/fear-related behavior and cortisol levels, while lowering locomotor performance. However, no significant impact was observed on social behavior (Gallas-Lopes et al, 2023).

In this study, the zebra fishes were divided into three groups, a control group, stress induced and probiotic treated group. After the probiotic treatment for 14 days, zebra fish from all the three groups were underwent T-Maze analysis which reveals their choice behavior and decision-making strategies. This behavioral test showed that probiotics treated group of zebra fishes had similar behavior while making choices while stress induced group had to take more time to select the path. This one behavioral test shows the positive impact of probiotics in a stress induced environment. The fishes were then sacrificed, and their tissues were collected for analysis. From the results it is found that probiotics increased SOD activity, converting superoxide radicals into less harmful molecules like hydrogen peroxide and oxygen. Increased CAT levels indicated improved protection against oxidative damage. Higher GPx levels suggest improved antioxidant defense against lipid peroxidation. Lower carbonyl levels indicated reduced protein oxidation and protection of cellular proteins from damage. Probiotic treatment also reduced lipid peroxidation and protected cell membranes from oxidative damage. If the probiotic-treated zebra fish show significant changes in the levels of these biomarkers compared to the control group, it suggests that probiotics have the potential to influence neurotransmitter balance, stress response, signaling pathways, mitochondrial function, and neurotrophic support in zebra fish. These results may indicate that probiotics have a beneficial impact on the neurobiology and overall health of zebra fish, potentially by modulating the gut microbiota and influencing various physiological pathways. The mRNA expression levels of various genes can provide insights into the molecular response to stress. GABA A and GABA B are subunits of the gamma-aminobutyric acid (GABA) receptor, an important inhibitory neurotransmitter in the central nervous system. Changes in GABA receptor expression can be indicative of alterations in the stress response and neuronal activity. CRF

(Corticotropin-releasing factor) is a key regulator of the hypothalamic-pituitary-adrenal (HPA) axis, which plays a central role in the stress response. Increased CRF expression is associated with elevated stress levels. Calcineurin is a calcium-dependent phosphatase involved in cellular signaling pathways. It has been implicated in stress responses, including those related to oxidative stress and neuronal plasticity. BDNF is a neurotrophin that promotes the survival and growth of neurons. It is involved in various processes, including stress adaptation, synaptic plasticity, and neurogenesis. Prohibitins are multifunctional proteins involved in cellular processes such as mitochondrial function, cell cycle regulation, and stress responses. PHB2, specifically, has been linked to oxidative stress and cellular protection mechanisms. By assessing the mRNA expression levels of these stress markers, researchers can gain insights into the molecular mechanisms underlying stress responses, neuronal activity, and cellular adaptation to stressors. These markers can help elucidate the impact of stress on biological systems and provide potential targets for therapeutic interventions. The probiotic-treated zebra fish showed significant changes in the levels of these biomarkers compared to the stress-induced group and this suggests that probiotics have the potential to influence neurotransmitter balance, stress response, signaling pathways, mitochondrial function, and neurotrophic support in zebra fish. These results may indicate that probiotics have a beneficial impact on the neurobiology and overall health of zebra fish, potentially by modulating the gut microbiota and influencing various physiological pathways. From overall findings of our study reveals that probiotic treatment significantly improved choice accuracy, spatial memory, decision-making strategies, and latency in zebra fish, indicating a positive impact on cognitive function. This supports the hypothesis that probiotics can influence cognitive abilities in other organisms, including humans.

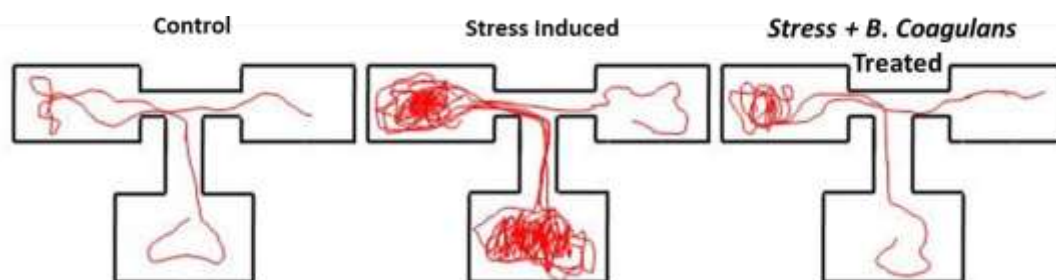


Figure 1. T- maze analysis of control and experimental group



**Figure 2 Histology analysis of control and experimental group**

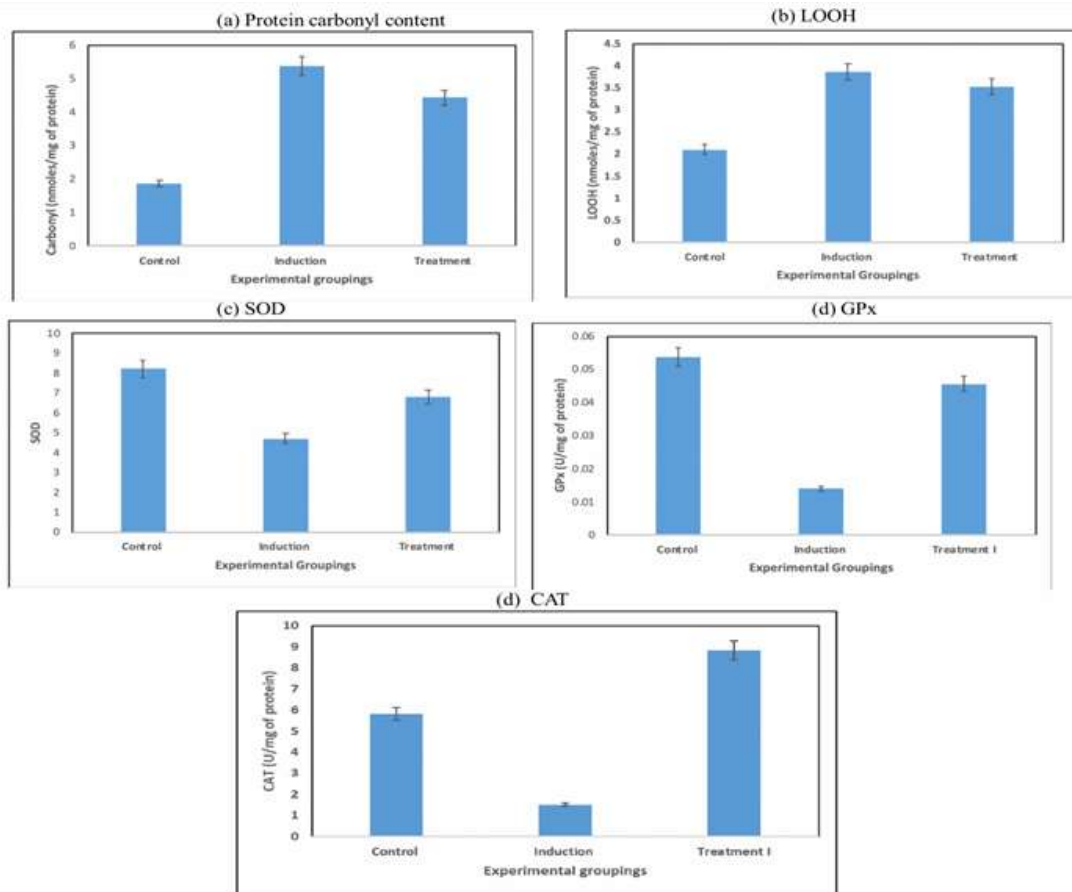


Figure 3 Biochemical assays of control and experimental group.

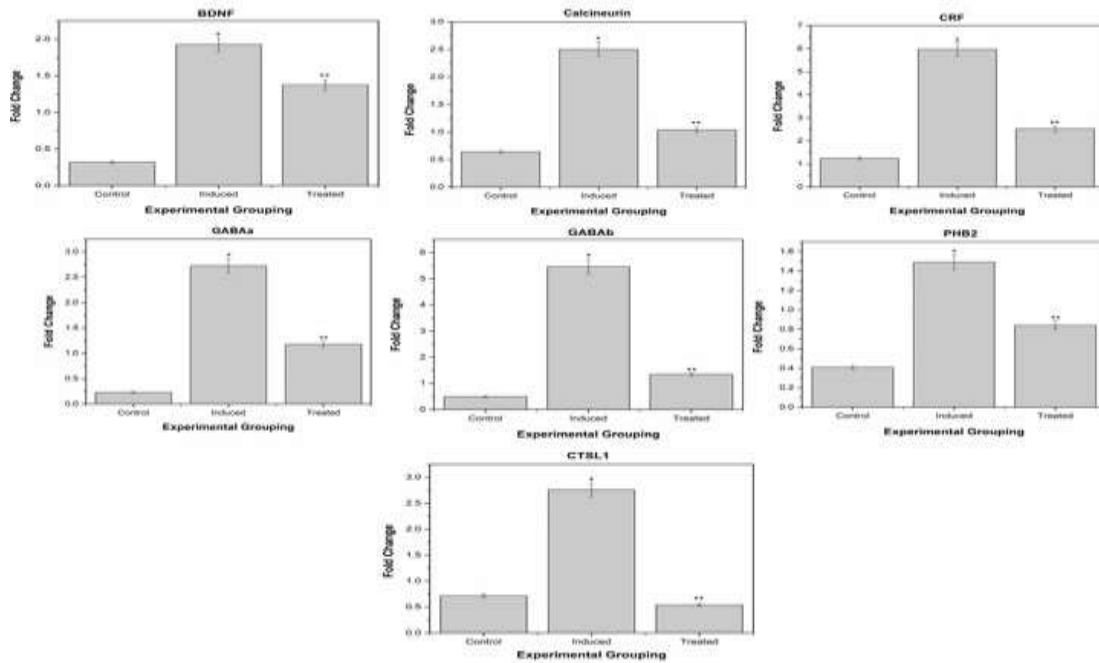


Figure 4 qPCR analysis of Biochemical marker analysis of control and experimental grouping Stress markers.

## Conclusions

Probiotics can modify zebra fish gut microbiota dysbiosis which affects physiological processes like stress response regulation. They improve intestinal health by enhancing the intestinal barrier, preventing harmful substances, and potentially regulating stress-related pathways and neurotransmitter systems. Overall, probiotics show promise as a potential approach for stress management in zebra fish. However, more research is needed to understand the specific probiotic strains, dosages, and mechanisms of action that can effectively modulate stress responses in zebra fish. These findings could have implications for understanding stress management in other organisms, including humans.

## Funding

The authors declare that no funds, grants or other support were received for this study.

## Conflict of interest

The authors declare no conflict of interest.

## References

1. Russell, G., Lightman, S. (2019). The human stress response. *Nat Rev Endocrinol* 15, 525–534. <https://doi.org/10.1038/s41574-019-0228-0>.
2. Nicolas Rohleder. (2019). Stress and inflammation – The need to address the gap in the transition between acute and chronic stress effects, *Psychoneuroendocrinology*, Volume 105, 2019, Pages 164-171, ISSN 0306-4530, <https://doi.org/10.1016/j.psyneuen.2019.02.021>.
3. Huang, Z., Xie, N., Illes, P., Di Virgilio, F., Ulrich, H., Semyanov, A., Verkhatsky, A., Sperlagh, B., Yu, S. G., Huang, C., & Tang, Y. (2021). From purines to purinergic signalling: molecular functions and human diseases. *Signal transduction and targeted therapy*, 6(1), 162. <https://doi.org/10.1038/s41392-021-00553-z>
4. Fontana, B. D., Cleal, M., Norton, W. H. J., & Parker, M. O. (2021). The impact of chronic unpredictable early-life stress (CUELS) on boldness and stress reactivity:

- Differential effects of stress duration and context of testing. *Physiology & behavior*, 240, 113526. <https://doi.org/10.1016/j.physbeh.2021.113526>
5. Sequeira-Cordero, A., Salas-Bastos, A., Fornaguera, J., & Brenes, J. C. (2019). Behavioural characterisation of chronic unpredictable stress based on ethologically relevant paradigms in rats. *Scientific reports*, 9(1), 17403. <https://doi.org/10.1038/s41598-019-53624-1>
  6. Friedman, Susan M. (2020). "Lifestyle (medicine) and healthy aging." *Clinics in geriatric medicine* 36.4, 645-653.
  7. Bremner, J. D., Moazzami, K., Wittbrodt, M. T., Nye, J. A., Lima, B. B., Gillespie, C. F., Rapaport, M. H., Pearce, B. D., Shah, A. J., & Vaccarino, V. (2020). Diet, Stress and Mental Health. *Nutrients*, 12(8), 2428. <https://doi.org/10.3390/nu12082428>
  8. Labanski, A., Langhorst, J., Engler, H., & Elsenbruch, S. (2020). Stress and the brain-gut axis in functional and chronic-inflammatory gastrointestinal diseases: A transdisciplinary challenge. *Psychoneuroendocrinology*, 111, 104501. <https://doi.org/10.1016/j.psyneuen.2019.104501>
  9. Molina-Torres, G., Rodriguez-Arrastia, M., Roman, P., Sanchez-Labraca, N., & Cardona, D. (2019). Stress and the gut microbiota-brain axis. *Behavioural Pharmacology*, 30, 187–200. doi:10.1097/fbp.0000000000000478.
  10. Sabit, H., Kassab, A., Alaa, D., Mohamed, S., Abdel-Ghany, S., Mansy, M., Said, O. A., Khalifa, M. A., Hafiz, H., & Abushady, A. M. (2023). The Effect of Probiotic Supplementation on the Gut-Brain Axis in Psychiatric Patients. *Current issues in molecular biology*, 45(5), 4080–4099. <https://doi.org/10.3390/cimb45050260>.
  11. Gubert, C., Kong, G., Renoir, T., & Hannan, A. J. (2020). Exercise, diet and stress as modulators of gut microbiota: Implications for neurodegenerative diseases. *Neurobiology of disease*, 134, 104621. <https://doi.org/10.1016/j.nbd.2019.104621>
  12. Musleh-Vega, S., Ojeda, J., & Vidal, P. M. (2022). Gut Microbiota-Brain Axis as a Potential Modulator of Psychological Stress after Spinal Cord Injury. *Biomedicines*, 10(4), 847. <https://doi.org/10.3390/biomedicines10040847>
  13. Sui, J., Jiang, R., Bustillo, J., & Calhoun, V. (2020). Neuroimaging-based Individualized Prediction of Cognition and Behavior for Mental Disorders and Health: Methods and Promises. *Biological psychiatry*, 88(11), 818–828. <https://doi.org/10.1016/j.biopsych.2020.02.016>

14. Marx, W., Lane, M., Hockey, M., Aslam, H., Berk, M., Walder, K., Borsini, A., Firth, J., Pariante, C. M., Berding, K., Cryan, J. F., Clarke, G., Craig, J. M., Su, K. P., Mischoulon, D., Gomez-Pinilla, F., Foster, J. A., Cani, P. D., Thuret, S., Staudacher, H. M., ... Jacka, F. N. (2021). Diet and depression: exploring the biological mechanisms of action. *Molecular psychiatry*, 26(1), 134–150. <https://doi.org/10.1038/s41380-020-00925-x>.
15. Liu, Q. F., Kim, H. M., Lim, S., Chung, M. J., Lim, C. Y., Koo, B. S., & Kang, S. S. (2020). Effect of probiotic administration on gut microbiota and depressive behaviors in mice. *Daru : journal of Faculty of Pharmacy, Tehran University of Medical Sciences*, 28(1), 181–189. <https://doi.org/10.1007/s40199-020-00329-w>
16. Ansari, F., Pourjafar, H., Tabrizi, A., & Homayouni, A. (2020). The Effects of Probiotics and Prebiotics on Mental Disorders: A Review on Depression, Anxiety, Alzheimer, and Autism Spectrum Disorders. *Current pharmaceutical biotechnology*, 21(7), 555–565. <https://doi.org/10.2174/1389201021666200107113812>
17. Zagórska A, Marcinkowska M, Jamrozik M, Wiśniowska B, Paśko P. From probiotics to psychobiotics - the gut-brain axis in psychiatric disorders. *Benef Microbes*. 2020 Dec 2;11(8):717- 732. doi: 10.3920/BM2020.0063. Epub 2020 Nov 16. PMID: 33191776.
18. Marttinen, M., Ala-Jaakkola, R., Laitila, A., & Lehtinen, M. J. (2020). Gut Microbiota, Probiotics and Physical Performance in Athletes and Physically Active Individuals. *Nutrients*, 12(10), 2936. <https://doi.org/10.3390/nu12102936>
19. Trigueiro, N. S. S., Canedo, A., Braga, D. L. S., Luchiari, A. C., & Rocha, T. L. (2020). Zebrafish as an Emerging Model System in the Global South: Two Decades of Research in Brazil. *Zebrafish*, 17(6), 412–425. <https://doi.org/10.1089/zeb.2020.1930>
20. Marcon, M., Mocelin, R., Benvenuti, R., Costa, T., Herrmann, A. P., de Oliveira, D. L., Koakoski, G., Barcellos, L. J. G., & Piato, A. (2018). Environmental enrichment modulates the response to chronic stress in zebrafish. *The Journal of experimental biology*, 221(Pt 4), jeb176735. <https://doi.org/10.1242/jeb.176735>
21. Kirsten, K., Pompermaier, A., Koakoski, G., Mendonça-Soares, S., da Costa, R. A., Maffi, V. C., Kreutz, L. C., & Barcellos, L. J. G. (2021). Acute and chronic stress differently alter the expression of cytokine and neuronal markers genes in zebrafish brain. *Stress (Amsterdam, Netherlands)*, 24(1), 107–112.

22. Kirsten, K., Pompermaier, A., Koakoski, G., Mendonça-Soares, S., da Costa, R. A., Maffi, V. C., Kreutz, L. C., & Barcellos, L. J. G. (2021). Acute and chronic stress differently alter the expression of cytokine and neuronal markers genes in zebrafish brain. *Stress (Amsterdam, Netherlands)*, 24(1), 107–112. <https://doi.org/10.1080/10253890.2020.1724947>.
23. Ngoc Hieu, B. T., Ngoc Anh, N. T., Audira, G., Juniardi, S., Liman, R. A. D., Villaflores, O. B., Lai, Y. H., Chen, J. R., Liang, S. T., Huang, J. C., & Hsiao, C. D. (2020). Development of a Modified Three-Day T-maze Protocol for Evaluating Learning and Memory Capacity of Adult Zebrafish. *International journal of molecular sciences*, 21(4), 1464. <https://doi.org/10.3390/ijms21041464>.
24. Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G., & Hoekstra, W. G. (1973). Selenium: biochemical role as a component of glutathione peroxidase. *Science (New York, N.Y.)*, 179(4073), 588–590. <https://doi.org/10.1126/science.179.4073.588>.
25. Chomczynski, P., & Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Analytical biochemistry*, 162(1), 156–159. <https://doi.org/10.1006/abio.1987.9999>.
26. Wong, M. I., Lau, I. H., Gordillo-Martinez, F., & Vasconcelos, R. O. (2022). The effect of time regime in noise exposure on the auditory system and behavioural stress in the zebrafish. *Scientific reports*, 12(1), 15353. <https://doi.org/10.1038/s41598-022-19573-y>.
27. Reis, C. G., Mocelin, R., Benvenuti, R., Marcon, M., Sachett, A., Herrmann, A. P., Elisabetsky, E., & Piato, A. (2020). Effects of N-acetylcysteine amide on anxiety and stress behavior in zebrafish. *Naunyn-Schmiedeberg's archives of pharmacology*, 393(4), 591–601. <https://doi.org/10.1007/s00210-019-01762-8>.
28. Gallas-Lopes, M., Bastos, L. M., Benvenuti, R., Panzenhagen, A. C., Piato, A., & Herrmann, A. P. (2023). Systematic review and meta-analysis of 10 years of unpredictable chronic stress in zebrafish. *Lab animal*, 52(10), 229–246. <https://doi.org/10.1038/s41684-023-01239-5>.