From Tank to Therapy: Using Zebrafish as an Alternative Model for Research in Depression

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Abstract. Depression, often known as major depressive disorder (MDD), affects 4.4% of the world's population and inflicts severe psychological, societal, and economic costs. Suicidal ideation, anhedonia, changes in food and sleeping patterns, cognitive deficits, and intense grief are some of these symptoms. The zebrafish has become a key tool for researching human brain problems, including depression, owing to its genetic tractability. Depression-like behaviours in zebrafish were modelled using behavioural tests such as the novel tank test. These tests also aid in evaluating antidepressant efficacy, making zebrafish a valuable tool in depression research. Zebrafish-based pharmacological research has investigated the effects of selective serotonin reuptake inhibitors, tricyclic antidepressants, monoamine oxidase inhibitors, and new antidepressant substances. These studies show that zebrafish have behavioural and neurochemical reactions to antidepressants similar to those observed in humans, making them an ideal model for studying the mechanisms of action and efficacy of these medications.

Keywords: Depression, Zebrafish, Neurobiology, Cortisol, Dopamine, Serotonin.

1. Introduction

Depression is a prevalent behavioural problem also called major depressive disorder which is characterised by recurring episodes throughout life, affects 4.4% of people worldwide and is connected to significant personal, social, and financial difficulties (Marwaha *et al.,* 2023 and Thapar *et al.,* 2022). Suicidal thoughts, anhedonia, irregular eating and sleep patterns, cognitive impairments, and extreme feelings of sadness and negativity are some of the symptoms of depression (Dobrek & Głowacka, 2023). Adolescence and childhood are high-risk times for mental illnesses, especially major depressive disorder having a big influence on people between the ages of 10 and 24 (Zhou *et al.,* 2020). An estimated 800,000 older individuals worldwide committed suicide as a result of depression, which is the main cause of disability worldwide. With incidence rates ranging from 10% to 20% across cultures, depression affects over 322 million people globally, necessitating more awareness and remedies (Bincy *et al.,* 2021). By 2030, major depression is estimated by the World Health

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Organisation to become the biggest cause of illness consequence (Blodgett *et al.,* 2021). As per the DSM-V, depressive disorders cover a variety of conditions such as major depressive disorder, dysthymia, premenstrual dysphoric disorder, disruptive mood dysregulation disorder, substance/medication-induced depressive disorder, depressive disorder resulting from another medical condition, and unspecified depressive disorder (Obuobi-Donkor *et al.,* 2021). The frequency of depression varies significantly by gender, with females experiencing the condition twice as often as males (Hayley *et al.,* 2021). Finding a single underlying reason for Major Depressive Disorder is challenging because of its multifaceted nature, which is impacted by social, psychological, and biological variables. Genetic, neurological, and neuroimaging components all affect the condition. Pharmacological, psychotherapeutic, and neuromodulator approaches are now used to treat MDD (Li *et al.,* 2021). The first line of treatment for depression is psychotherapy and antidepressants, and both are beneficial. While other kinds of psychotherapy for depression have also undergone significant testing, cognitive behavioural therapy (CBT) has been the subject of the most research. These include psychological activation, life review, solving issues, relational, the psychodynamic approach, and "third wave" treatments as well as non-directive supportive counselling (Cuijpers *et al.,* 2020).

2. Etiology of depression

2.1 Depression and Early Life Trauma

With 3 million reports and 1 million confirmed cases each year, childhood trauma is a common phenomenon. It is typical to experience 60% neglect, 20% physical abuse, and 10% sexual abuse. In addition to physical violence, 8–9% of men and 20–25% of women claim to have experienced sexual abuse (Horowitz *et al.,*1997). If it is more common, emotional abuse and neglect may be more difficult to quantify. Trauma raises the likelihood of substance abuse, (post-traumatic stress disorder) PTSD, and mood disorders. Women who experienced abuse as children are four times as likely to have depression. Resilience elements may be impacted by the timing of adversity and include positive parental care, personality, and relationships. Effective interventions require more research on sensitive times and resilience (Bremner *et al.,* 1993).

2.2 Environmental-Gene Interactions

This article presents a solid overview of the complex interplay between genetics and early trauma in the development of depression. The authors highlight the role of specific genes such as CRHR1, SERT, FKBP5, and BDNF, and discuss how variations in these genes can influence an individual's susceptibility to depression following traumatic experiences. They also emphasize the importance of considering gene-to-gene interactions and the involvement of neurobiological pathways such as CRF and HPA axis dysfunction (Caspi *et al.,* 2003). According to studies, FKBP5 SNPs and childhood maltreatment int. The findings underscore the need for a personalized approach to depression treatment, taking into account an individual's genetic makeup and early life experiences. Overall, this article provides valuable insights into the intricate mechanisms underlying depression and the potential for genetic testing to guide more effective interventions (Karg *et al.,* 2011).

2.3 Serotonin Alterations in Depression

The serotonin system is absolutely essential for regulating mood. Extensive research has consistently shown that individuals with depression typically exhibit lower levels of serotonin in their brains and possess fewer serotonin transporter binding sites, which significantly impact serotonin levels. Consequently, this can significantly hinder the effectiveness of antidepressant medications. Moreover, individuals with specific variations of the SERT gene have a higher predisposition to experiencing depression, particularly in the presence of childhood trauma.

One study found that people with a specific variation of the SERT gene were 17 times more likely to develop depression if they had experienced childhood trauma. This suggests that genetics and environment can interact to increase the risk of depression.

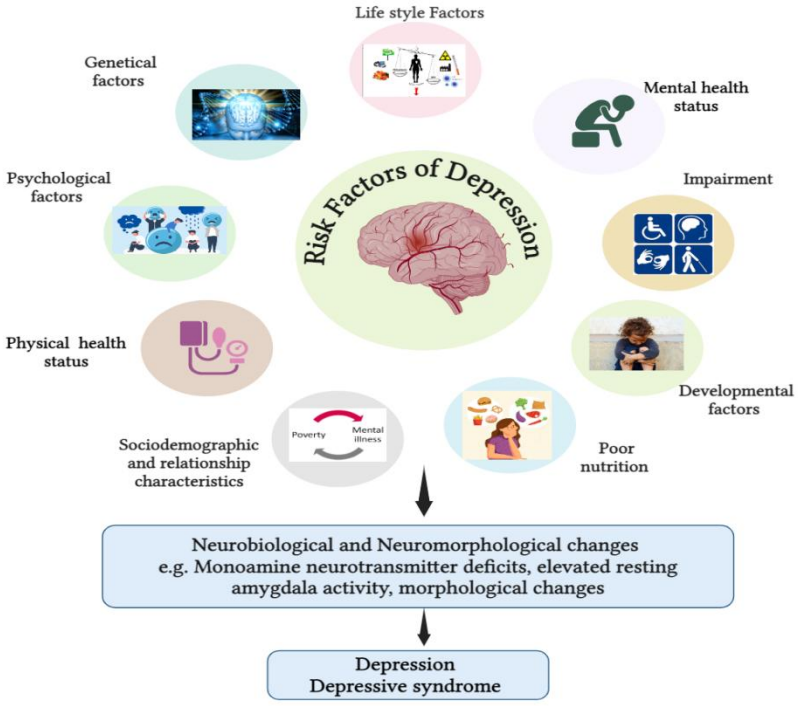

Fig: 1. Risk Factors of Depression

3. Zebrafish general characteristics

The zebrafish, Danio rerio, has recently gained attention as a powerful animal model for studying various human brain disorders. One of the most common fish species in small lakes and paddy fields is the zebrafish, an aquatic fish native to Southeast Asian nations (Spence *et al.,* 2008). Zebrafish are a small, cost-effective aquatic vertebrate species with close genetic and physiological similarities to humans, making them valuable for research (Kalueff *et al.,* 2014).

zebrafish provide several advantages over rat models in preclinical drug discovery and screening, Zebrafish are useful to model organisms for biological research because of their (i) genetic tractability, (ii) small size of both the larval and adult stages, (iii) easy maintenance and housing, (iv) relatively quick fertilization and development, (v) translucent embryos, and (vi) stable reproductive capacities in controlled laboratories. Adults who can reproduce after the age of three months, for example (Alsop & Vijayan *et al.,* 2008), (Gerlai, 2010),(Singleman & Holtzman, 2014). The fully sequenced zebrafish genome has orthologous genes corresponding to about 82% of disease-related genes found in humans (Schier *et al.,* 2013). Modeling human conditions in zebrafish enables the discovery of potential therapeutic targets and the understanding of the underlying molecular interactions (Vaccaro *et al.,* 2012). It is also a valuable model for understanding and finding potential therapies for human metabolic diseases (Santoro *et al.,* 2014). The diencephalon and telencephalon are also present in the fore-, mid-, and hindbrain regions of zebrafish brains, which are analogous to mammalian brains in general and human main neurotransmitter activity in particular(Panula *et al.,* 2006).

All of the neurotransmitter systems present in mammals, such as the dopaminergic, serotonergic, cholinergic, and non-adrenergic systems, have been identified in zebrafish(Agostini *et al.,* 2018). Zebrafish have significant cognitive ability and wide decision-making capabilities, in addition to being very responsive to pharmacological intervention (Oliveira *et al.,*2013). The results support the use of zebrafish as a model for studying depression has provided valuable insights into the neurobiological mechanisms associated with this complex disorder.

4. Depression research in zebrafish

Zebrafish *(Danio rerio)* have emerged as a vital model organism in neuropsychiatric research, particularly for investigating depression and evaluating new antidepressants. This work cover a wide range of depression-related topics, from behavioral analysis to genetic and pharmaceutical research. Here is a review of the completed depression-related zebcoversh studies:

4.1 Behavioral Studies

4.1.1 Novel tank test

Zebrafish depression-like behaviours are frequently measured using the novel tank test (NTT) (Ngadni *et al.,* 2021). A common approach for evaluating the effects of stressful interventions on zebrafish is the novel tank test. For a minimum of 20 days before the novel tank testing, they were kept in 40-litre glass tanks with filtered facility water (pH of 7.0–7.5 and room temperature of 25–27 °C) (Cachat *et al.,* 2010). Zebrafish are normally placed in an unfamiliar, small rectangular tank that is roughly divided into two equal horizontal halves during the NTT procedure, and they are allowed to swim freely for five to thirty minutes (Grossman *et al.,* 2010). Danio rerio spends most of their time in the lower portion of the tank, or the "protection zone," when they are placed in a fresh environment. Once they get used to the new surroundings, they explore the area (Meshalkina *et al.,* 2017). The time spent in the upper and lower parts of the tank, the number of entries into the upper part, the delay in

entering the upper part, the freezing time, the irregular movements, the immobility, the swimming speed, and the distance travelled are the most significant parameters recorded in the NTT that characterize the depression-like behaviour of the tested subjects (Nguyen *et al.,* 2014 and Haghani *et al.,* 2019). To capture the activity of the test fish, a video camera is positioned in front of the tank. Noldus an automatic video-tracking program, is used to evaluate digital recordings (Qin *et al.,* 2014). In a tank, distinct swimming zones are typically marked out to track how much time is spent in each area (Stewart *et al.,* 2010)

4.1.2 Light or dark box test

The light/dark paradigm is another behavioural test used on zebrafish, which is derived from rodents. The inherent tension between exploring a new location (an illuminated zone) and remaining in a secure environment (i.e., a dark zone) serves as the basis for this test. The current version of the light/dark test for zebrafish was first put forth by Serra *et al*. (1999) and then verified by Maximino and associates as a behavioural test to assess anxiety-like responses in tiny teleosts (Maximino *et al.,* 2007), (Serra, Medalha, & Mattioli, 1999). The light/dark box apparatus is made up of two equal-sized chambers divided by a rectangular plastic tank of L30 cm by W15 cm by H15 cm, with the water level fixed at H13 cm. Transparent material was used in place of the white section, and the top and sides of the tank were covered to create the dark compartment. For five minutes, adult zebrafish were subjected to the light/dark box test. A camera positioned above the light/dark box equipment recorded each behavioural session, to prevent any interference with the behavioural reactions, the experimenter remained outside the testing room during the recording. A stopwatch and digital counter were used to manually count the parameters on the films after they had been examined by human eyes. The total number of crossings between the compartments and the total amount of time (s) spent inside each compartment were the two measures that were measured. Percentage of total time spent in each compartment was used to report zone preference (Champagne *et al.,* 2010 and Maximino *et al.,* 2012).

4.1.3 Social preference test

Social preference in zebrafish is their inclination to live near members of their species (Liu *et al.,* 2016). By monitoring how individuals react to or interact with social stimuli, including the presence of other members of the same species, social behaviour in zebrafish is investigated (Pham *et al.,* 2012). A standard social preference test could comprise five 10 × 10 cm cubicles separated into a 50-cm Plexiglas hallway. The target fish is placed in an exposure chamber that is divided from the remaining parts of the equipment by a transparent divider. Fish used in experiments are pre-exposed for 20 minutes to either drug or drug-free water (control) (Lachlan *et al.,* 1998). Zebrafish that have been exposed to drugs or which are not exposed to drugs i.e. control group are individually delivered into the apparatus' middle zone, temporarily separating it from the two arms of the corridor. After the first 30-second acclimatization period, the two sliding dividers should be gently raised, allowing the zebrafish to spend the next six minutes exploring the device (Robert, 2017). The amount of time spent in the centre, in nonspecific arms, or empty arms, as well as the number of entries made, can be used to manually assess fish behaviour using video-tracking software. Based on this information, the ratios of nonspecific: empty arm entries and the corresponding duration ratios can also be computed (Stowers *et al*., 2017).

4.1.4 Open field test

A common technique for evaluating zebrafish behaviour and motility is the open field test, which may also be used to assess aversion and anxiety-like reactions by looking at changes in location preference. In an infrasound speaker-equipped testing arena, zebrafish behaviour and movement were evaluated in multiple virtual zones. The open field test arena is a plastic tank measuring 28 cm by 28 cm and measuring 15 cm in height. Three virtual zones were created in the arena: Zone1 (Speaker), Zone 2 (Middle), and Zone 3 (Far). Additionally, a 4.8 cm thigmotaxis zone is created from the inside perimeter (Scatterty *et al.,* 2023 and Karlsen *et al.,* 2004). While time spent in the outer zone (transparent zone) had significantly increased over time, the behavioural pattern of numerous squares transverse, distance travelled, and high velocity had significantly decreased by the end of the 5-min test session, suggesting that zebrafish are capable of acclimatization, a type of non-associative learning (Sousa *et al.,* 2006 and Johnson & Hamilton, *et al.,*2017). Fish with strong dark aversion (SDA) qualities have stronger thigmotaxis (desire for the walls) than fish with variable dark aversion (VDA) traits. (Engeszer *et al.,* 2007).

Table 1: Zebrafish Behavioral Analysis

TEST	PARAMETER	OBSERVATION
Novel tank test	Video recordings were made for six minutes (s) to capture the latency, duration, frequency of entrances in the upper section of the container, number of freezes, and time spent immobile.	The degree of **"bottom-dwelling,"** and the limitation of exploratory behavior are markers of depression-like behavior. Variation in the swimming velocity.
Light or dark box test	For five minutes, the zones of preference, crossings between the compartments, and the amount of time spent in each compartment were video recorded.	**Increase** negative phototaxis (Dark versus bright section preference)
Social preference test	Used to observe the response of zebrafish to conspecific, heterospecific, or both types of changes in coloring and patterning	**Decreased** interactions with other fishes. Delay to shoal cohesiveness, social distance, and time apart from the group.

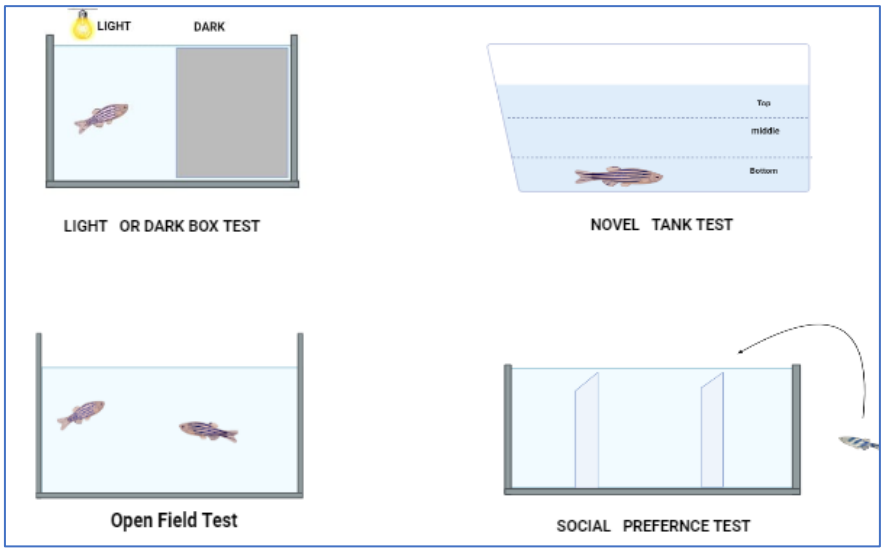

Fig. 2 Models to evaluate depression like behaviour in adult zebrafish.

5. Conclusion

In conclusion, zebrafish *(Danio rerio)* serve as a valuable model organism for investigating depression due to their genetic homology with humans, ease of genetic manipulation, and ability to exhibit behaviours analogous to human depressive symptoms. Behavioural assays such as the novel tank test, light/dark box test, and social preference test provide robust platforms for studying depression-related behaviours in zebrafish. Pharmacological studies utilizing selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), and novel compounds have elucidated mechanisms of action and efficacy in alleviating depression-like behaviours. Moreover, genetic studies focusing on gene-environment interactions and neurobiological investigations into neurotransmitter systems (e.g., serotonin, dopamine) provide critical insights into the pathophysiology of depression. These findings underscore the translational relevance of zebrafish models in advancing our understanding of depression, facilitating drug discovery, and potentially guiding therapeutic interventions for this complex psychiatric disorder. Further research utilizing zebrafish holds promise for uncovering new treatment strategies and enhancing clinical outcomes in depression management. These findings highlight the translational importance of zebrafish models in improving our understanding of depression, aiding drug discovery, and potentially guiding treatment strategies for this complex mental condition. Further study with zebrafish has the potential for discovering novel therapeutic techniques and improving clinical outcomes in depression management.

6. Declarations

Competing Interests: On behalf of all authors, the corresponding author states that there are no Competing interests.

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