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FROM TO SERENITY: ZEBRAFISH AS A MODEL SYSTEM FOR ANXIOLYTIC INVESTIGATIONS

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Abstract

7–10% of individuals throughout the world suffer from one of the prevalent mental diseases known as anxiety disorders. An excellent animal model for comprehending anxiety-related behavior is the zebrafish. They are most commonly employed to test different anxiety medications and investigate the neurological, physiological, and genetic causes of anxiety. To study and analyses the zebrafish model for anxiolytic activity utilities, benefits in comparison animal and other delicious facts about zebrafish model. We have looked through the literature to find several techniques for inducing anxiety in adult zebrafish using physical (like use of light and temperature, vibration or "tapping, Restrain, magnetic stress, shallow water etc.), mechanical (through glass rods to disturbing swimming behavior, separation, etc.), and chemical stimuli (induce anxiety though anxious chemical like Benzo[a]pyrene, alarm substance etc.). This review also focuses on finding the best ways to make adult zebrafish anxious without significant causality and at a reasonable price. In this review, we also explain these processes that someone common pathology followed. This analysis also covers some of the most popular conventional drugs and herbal anxiety remedies and some popular standard drugs use in market. In the last we explained some specific and commonly used behavioral model for observing behavioral parameter of anxiety. In Summary, zebrafish model are very demanding and most specific model to investigate anxiety like behavior and anxiety evoking for chemical induced anxiety, environmental stressors, anxiety inducing drugs, including novelty, and environmental toxin, mechanical stressor. This review was aimed to evaluate anxiety potential in a zebrafish model by induction by different stressor, which included neuro pharmacological evaluation to determine behavioral parameters in the novel tank diving test (NTDT), light-dark test (LDT), and Open field test. Similar to cortisol, anxiety-related neuroendocrine responses in zebrafish are likewise robust, responsive, and significantly (and symmetrically) correlated with behavioral outcomes. Overall, data demonstrates the reliability and effectiveness of the adult zebrafish model for researching both acute and long-term anxiety-like states.

Keywords: - Zebrafish, Anxiety, Stressor, Plant drug, Behavioral parameter.

Introduction:-

Zebrafish provide unique advantages for high-throughput pharmacological screening due to their cost-effectiveness and experimental convenience **(Giacomotto and Ségalat, 2010) [1].** Being physiologically similar to humans and a relatively simpler vertebrate species, zebrafish allow researchers to study pathways and processes related to human etiology and therapeutic approaches **(Shin, F. et al., 2017) [2].**These fish species possess all the 'classical' vertebrate neurotransmitters and exhibit potent responses to stress in their neuroendocrine system **(Mueller & Vernier, et al., 2004)[3].** Neurotransmitter systems, including GABA, dopamine, and serotonin, begin functioning during zebrafish larvae's origin and development **(Alsop & Vijayan, et al., 2008) [4].** Increasingly, the zebrafish (Danio rerio) has gained attention as an animal model for various human brain-related issues **(Kalueff & Echevarria, et al., 2014) [5].** Zebrafish, a small aquatic vertebrate species, bears a remarkable level of similarity to humans in physical, physiological, and genetic aspects **(Kalueff & Stewart et al., 2014) [6].** Around 82% of disease-related human genes have comparative similarities within the fully sequenced genome of the zebrafish **(Howe et al., 2013) [7].** The central nervous system (CNS) of zebrafish exhibits similar structures and characteristics to mammals in terms of neuroanatomy, cellular morphology, and overall structure **(Kalueff & Stewart et al., 2014) [6].**

A tiny aquatic vertebrate species known as the zebrafish has a significant degree of physical, physiological, and genetic similarity to humans. **(Kalueff & Stewart et al., 2014)[6**] And also compare as other in gene level about 82% of disease-related human genes comparative similar in the completely sequenced genome in zebrafish. **(Howe et al., 2013)[7]** The zebrafish CNS resembles mammals in terms of overall structure, neuroanatomical characteristics, and cellular morphology. **(Kalueff & Stewart et al., 2014)[6]** Researchers have found an amygdala-like structure within the medial teleost pallium **(Von & Trotha et al., 2014)** [**8**]. In human brain anatomy, emotions and affect absorption are significantly reliant on the amygdala. Clinical anxiety often results in pathological over activity of this structure **(Richendrfer & Pelkowski, 2012) [9]**. Studies highlight how similar brain mechanisms are involved in social-related anxiety issues (**Furmark & Tillfors et al., 2004)** [**10]** and abuse of drugs also **(Buffalari and See, 2010). [11]** The majority of behavioral anxiety models in zebrafish have been adopted from proven rat/mice models, in which stressed stimuli, including like new environments, alarm pheromones, light and dark spaces, and pictures or dummies of potential natural predators, are displayed to zebrafish. **(Cianca & Bartolini et al., 2013) [12]** Moreover, Zebrafish are an excellent animal model for scientific study because they are affordable, low-liability, & offspring in large numbers diseases and disorders. **(Gerlai & Blaser et al., 2006)[13].**

Anxiety is characterized as a prolonged emotional state triggered by a situation that may seem threatening, yet offers minimal or no likelihood of actual harm occurring. **(Takagi & Sakai, et al., 2018) [14].** the lifetime incidence of anxiety disorders is 30%, making them the most common type of serious mental illness. **(Kesslera RC. et al., 2007)[15].** Panic attacks disorder (PADs), post - traumatics stress disorder (PTSDs), generalized anxiety disorder (GADS), and particular phobias (Pbs) are a few of the several forms of anxiety disorders. **(American Psychiatric Association, 2013)[16**] SSRIs and cognitive behavioral therapy (CBT) are often the initial treatment options for anxiety disorders. Patients who do not react to these therapies are subsequently prescribed tricyclic antidepressants or SNRIs. **(Bystritskys A. et al., 2006) [17**]. so that in zebrafish the novel diving tank test, signs of anxiety in adult fish may involve a reluctance to explore the upper area or a stronger preference to remain at the bottom. **(Stewart & Gaikwad et al., 2012) [18].** Zebrafish are given the opportunity to explore both the brightly illuminated and dimly lit sections in the light-dark test. When zebrafish prefer spending more time in the dark section, it signifies an anxiety-like response, which can be influenced in either direction by anxiolytic or antigenic treatments (**Kalueffs & Gebhardt et al., 2013).[19**] High-throughput anxiolytic drug screening is especially suitable for use in the zebrafish model. (**Lundegaard et al., 2015) [20**] Sedatives are typically used to treat anxiety disorders. They primarily modulate the histaminergic, GABAergic, and adrenergic systems to elicit anxiety reduction, and drowsiness. **(Koob et al., 1992)[21]**. Zebrafish $GABA_A$ and $GABA_B$ receptor subunits, as well as

the histamine H_1 receptor, are comparable to those found in mammals. **(Renier & Faraco et al., 2007)[22]** Zebrafish is increasingly used in medication screening for the CNS, and translational research on stress and anxiety. (**MacRaea & Peterson, et al., 2015) [23]**

Although anxiety affects people of all ages, it is more prevalent and incapacitating in older people and Over 40 million persons in the US struggle with anxiety problems **(Alipour & Sajadi et al., 2009) [24].** Anxiety disorders affect 3–15 percent of individuals and are prevalent in the elderly, especially those with chronic conditions, with a prevalence of anxiety symptoms in the elderly of 15–52 percent.(Ryan & Scali et al., 2011)[45] According to the 4-meta-analytic evaluations, among adults, social phobia (SPS) (20%), GADs (13%–20%), and PADs (17%–22%) have the greatest lifetime rates, followed by PTSD (11%–17%), social anxiety disorder (13%), OCD (10%–13%), specific phobias (11%), and agoraphobia (8%). **(Yapici & Kacar et al., 2018)[25].** this disorder is the most prevalent mental condition among adults in the U.S., with a lifetime prevalence rate estimated to be 28.8%. (**Kessler & Berglund et al., 2005).[26]** Anxiety disorder have a yearly medical cost of 42.3 - 46.6 billion dollars in the U.S. in addition to having negative immediate and long-term health implications. (**DuPont et al., 1996)[27].** Anxiety disorders often exhibit similar symptoms, such as a subjective feeling of unease, which can lead to challenges with sleep, concentration, social interactions, and work-related tasks. Momentarily diverting your thoughts might provide a temporary break from the underlying feelings of anxiety. This situation is common among individuals with panic disorder, marked by brief episodes of intense fear and a feeling of impending doom, accompanied by physical symptoms like chest pain, dizziness, and breathlessness. **(Markowitz et al., 1989)[28].**

Pathology:

Anxiety research has focused on exploring the brain and endocrine systems associated with anxiety to better understand the pathophysiology and therapeutic options for stress-related diseases **(Aponte, p. et al., 2019) [29].**The amygdale, hippocampus, and other brain regions are in responsibility for regulating and balancing the effects of stress. **(McEwen, 2007)[30]** The hypothalamic-pituitary-adrenal (HPA) axis and autonomic nerve system play crucial roles in controlling stress reactions. Anxiety and other stress-related illnesses are linked to changes in the HPA axis and hypothalamic structure and/or function. **(Faravellia C & Sauro, et al., 2012)[31]** Zebrafish have comparable human homologs in their central nervous system (CNS), genetic sequences, and metabolic pathways, which promotes the use of zebrafish as an animal model for research into the neural mechanism underlying behavior **(Guo S, 2009)[32].** In terms of stress studies, zebrafish have well preserved neural and endocrine systems that control biological and behavioral responses to stress. **(Stewart et al., 2012)[33]** For instance, the zebrafish hypothalamic-pituitary-interrenal (HPI) axis is comparable to the mammalian HPA-axis **(Nesan D & Vijayan M, 2013) [34]** much like mammals. Zebrafish release corticotropin-releasing hormone (CRH) from their hypothalamus when they are under stress. This, in turn, triggers the anterior pituitary to release adrenocorticotropic hormone (ACTH). The interrenal cells are stimulated by ACTH to produce and release cortisol into the bloodstream. **(Alsop & Vijayan, 2009) [35]** and the result responsible for anxiety. Additionally, ongoing stress causes pathological alterations in gene expression and brain activity that disrupt a number of biological processes. **(McEwen, 2017) [36].** Stress plays a substantial role in the pathophysiology and etiology of affective disorders, impacting the hypothalamic-pituitary-adrenal (HPA) axis and glucocorticoid receptor (GR) feedback, ultimately leading to the excessive release of glucocorticoids such as cortisol. **(Weiner & Harvey et al., 2017) [37].** the latter, in consequence, may penetrate the brain and activate neuroglia, which would alter normal brain circuits and aggravate emotional pathogenesis by causing neuro inflammation and apoptosis. **(Munhoz & Sorrells, 2010) [38**]. similarly, interleukin (IL)-1, IL-6, and tumor necrosis factor-alpha (TNF-α) levels in the blood are frequently raised in stressed (e.g., PTSD) individuals and are related to both the length and degree of symptom severity **(Smith & Kassem, et al., 2019) [39].**

In terms of treatment-based pathophysiology, the involvement of neurotransmitter systems, including the GABAergic, serotonergic, and noradrenergic systems, plays a crucial role. A reduction in GABA conduction is associated with anxiety in humans **(Ströhle & Gensichen, et al., 2018) [40**]. The deficiency of cortical GABAA receptors is linked to the severity of anxiety symptoms in panic disorder **(Telles C. & Saraiva, 2018) [41].**

Furthermore, selective serotonin reuptake inhibitors (SSRIs) have shown efficacy in the treatment of anxiety (**Edinoff & Akuly et al., 2021) [42].**

Moreover zebrafish models can be used to study a variety of diseases and disorders, primarily neuronal diseases, and to investigation things like the regulation of metabolic disorders, intestinal protection, renal protection, anti-osteoporosis, anti-melanogenesis, and safety measurement. **(Lin & Zhuang, et al., 2021) [43**] Zebrafish exhibit all the genes related to GABAA and GABAB receptors, including those responsible for encoding proteins associated with the GABAA receptor **(Bandara S. & Carty, et al., 2020) [44].**

Numerous stressors (e.g., social, physical, and chemical) can alter physiological and cellular stress indicators, such as exposure to neighborhood violence. **(Theall et al., 2017) [45]**

Physical or mechanical stressor

Fish exhibit robust stress reactions triggered by physical stimuli **(Perry R. & Salama, 1996) [46].** Notably, physical stresses are primarily processed by the mammalian brainstem and hypothalamus. The paraventricular nucleus (PVN) of the hypothalamus manages the activation or inhibition of the HPA axis, a process subsequently triggered through the brainstem **(Ulrich-Lai & Herman, et al., 2009) [47].** Moreover, additional brain regions involved in regulating autonomic stress responses are activated by physical stresses, including the dorso medial hypothalamus (DMH) and nucleus of the solitary tract (NTS) **(Geerling & Ulrich et al., 2010) [48].** The amygdala, hippocampus, and prefrontal cortex (PFC) are other critical brain regions engaged in physical stresses, receiving inputs from cortical and subcortical areas and funneling their outputs to subcortical relay sites, allowing for the downstream processing of limbic information. **(Ulrich-Lai and Herman, 2009) [47**] Chasing is an example of a physical stressor that has been utilized as a standard stressor **(Giacomini Ac & Abreu Ms 2016), [49]** additionally, spatial limitation is employed as a stress model for zebrafish behavioral evaluation **(Piato & Rosemberg, et al., 2011) [50].** Other types of physical stressors are also used to induce anxiety in adult zebrafish, such as 4-day acute thermal treatments. These treatments may increase anxiety-like behaviors at 18°C compared to 26°C or 34°C, resulting in down regulated anxiety-related protein signaling pathways **(Nonnis & Angiulli et al., 2021) [51]** The influence of a high-fat diet (HFD) on zebrafish resulted in amplified aggression in the mirror-induced aggression test and anxiety-like behaviors in the novel tank diving test **(Picolo & Quadros, et al., 2021)[52].** We can deliver a visual and lateral line stimulus (vibration or 'tapping') by stress apparatus to the fish by moving an object, such as a ball colliding with the side glass of the experimental tank, and we can also present data on how zebrafish respond to the tapping. Some models or apparatus are also used to elicit fear or anxiety responses in adult zebrafish. **(Ro & Noronha et al., 2022) [53]** Acute restraint stress activates cortisol-releasing hormones (CRH) and increases cortisol levels, which are responsible for anxiety in zebrafish as observed in novel diving and locomotor activities tests. **(Ghisleni & Capiotti et al., 2012)[54]** Social isolation in adult zebrafish affect cortisol and dopamine level in brain that responsible for anxiety. **(Von K. & Sorensen et al., 2010)[55**] Exposing zebrafish to short-term stress, such as 90 minutes of exposure to various stimuli like cold water, bright light, swirling water, shallow conditions, and physical restraint, can increase the activity of specific genes in the brain that are associated with stress. This increased gene activity is believed to contribute to anxiety in adult zebrafish **(Yang & Wang, 2020) [56].** Another stress-inducing method, Unpredictable Chronic Stress (UCS), involved a two-week protocol that included factors like restraint stress, elevating tank water temperature to 33°C, social isolation, altering tank conditions, and exposing the fish to potential predators.**(Piato & Capiotti, et al., 2020)[57]** Neuropeptide Y deficiency Zebrafish induce anxiety-like behaviours through acute stress (like cold stress (10 °C for 2 s) **(Shiozaki & Kawabe et al.,2020)[58**]. Studies indicate that predictable chronic stress, along with conspecific alarm substances, chasing the fish with net and immediate effects of repeated stressors, can provoke anxiety in zebrafish **(Quadros & Rosa, 2021) [59].** Prolonged chronic unpredictable stress (PCUS) modified both behavioral patterns and neurochemical levels, particularly affecting norepinephrine, and significantly induced severe anxiety in the zebrafish **(Demin & Kolesnikova et al., 2021) [60]**. Additionally, the observations revealed that fluoxetine and EPA (eicosapentaenoic acid) were effective in restoring norepinephrine levels, while LPS (lipopolysaccharide) and EPA increased dopamine levels **(Ovchenkov & Chareev et al., 2020) [61].**

Table 2: Physical or Mechanical stress induces anxiety in Adult Zebrafish and observational parameter.

S/N	Research title	Methodology	Pathology	Behaviour models	Biochemical	References
1.	**Variation temperature**	On Temperature 18^0C zebrafish show more potential anxiety like behavior in comparison 26^0C, 34^0C,	Due temperature variation stress responsible to reduced expression of synaptic proteins and down regulated protein pathways result produces anxiety-like status of zebrafish.	NDT & LAD test, Social preference test, Mirror test, Y-Maze test (YMT).	Proteomic analysis	Nonnis & Angiulli et al., 2021 [51]
2.	**Tapping Assay**	The method delivers a vibration or "tapping" to zebrafish to create fear in zebrafish with the help of a test tank.	Trapping or vibration method to that creates fear in zebrafish responsible for stress that activated HPI-axis pathway.	The tank test, Video-tracking software, Observation - through JVC GZ-MG_{50}U Digital video camera.		Noronha, & Mirza, et al., 2022 [53]
3.	**Restraint stress**	Acute restraint stress is created when animals are enclosed in micro centrifuge plastic tubes (2 ml) with small openings in both ends.	HPI - axis, governing cortisol levels and Activation this system starts in the hypothalamus, managing the intricate signaling involved in the neurobiology of stress and its stressful cues.	Novel Diving test and also observe locomotors activity ANY-Maze® recording software	cortisol	Ghisleni & Capiotti et al., 2012[54]
4.	**Social interactions**	Zebrafish isolated for a week but placed in an enriched environment for 15-min to develop stress.	Social isolation exhibit increased cell proliferation in the telencephalon and raised cortisol levels across the entire body via the HPI (hypothalamic-pituitary-interrenal) axis.	open field test light and dark test	cortisol brain dopamine level	von Krogh et al., 2010[55]
5.	**Acute combined stress**	Cold (19 ℃) water , bright 1200-lx light, Overtaxing at 650 rpm (magnetic stirrer), Shallow (0.5-cm deep) water, Restraint stress.	The stress-induced alteration in the HPA-axis disrupts the feedback mechanism of the glucocorticoid receptor (GR), resulting in an excessive release of glucocorticoids, which contributes to anxiety. .	Novel and diving test	Cortisol	Yang & Wang et al., 2020[56]
6.	**chronic Unpredictable stress**	Restraint stress, Social isolation, Crowding, Low water level, Cooling, Tank change, Heating Cooling Predator, Chasing	A stressful signal stimulates the secretion of hypothalamic (CRF) hormones that activate HPI and release cortisol, which is responsible for anxiety-like behaviour in zebrafish.	Locomotor activity, color change test, Shoal cohesion, avoidance protocol,	Corticotrophin-releasing factor (CRF) Glucocorticoid receptor (GR), Cortisol	Piato & Capiotti et al., 2011[57]
7.	**Neuropeptide Y factor**	The fish underwent sudden cold stress (10 °C for 2 seconds) and were subsequently transferred to the mirror test tank.	Acute cold water treatment affects neuropeptide Y regulation, which is responsible for anxiety.	Mirror test light and dark test	Immunohistochemistry mRNA expression levels	Shiozaki, et al., 2020[58]
8.	**Predictable chronic stress**	Predictable chronic stress with conspecific alarm substances, net chasing,	Cortisol, the hormone that causes anxiety, is produced and	Novel Diving test , Light and dark test	cortisol test	Quadros & Rosa et al., 2021[59]

		Acute effects of the homotypic stressors	released when the HPI axis in teleost fishes is activated.			
9.	**Chronic unpredictable early-life stress (CUELS)**	Changes in light/dark cycles, social isolation, overcrowding, water changes, temperature alterations (cooling/ heating), mechanical stirring, and immersion in shallow water twice a day were conducted for a period of 14 days.	Cortisol levels were evaluated to assess the effect of CUELS on the HPI-axis.	Novel Diving, Light and dark test, open field test, Pavlovian fear conditioning FMP Y-maze test, Shoaling test.	Whole-body cortisol	**Fontana, & Gibbon et al., 2020 [61]**
10	**Prolonged chronic unpredictable stress model (**PCUS)	PCUS model lasting 11 weeks was implemented in zebrafish to more accurately replicate chronic stress experienced by human populations.	PCUS alter behavioral and neurochemical (norepinephrine levels) in zebrafsh, and induced severe type of anxiety.	Novel tank test, Shoaling test, Light and dark test ,	Norepinephrine Dopamine serotonin 3,4-di hydroxyphenyl acetic acid, 5-hydroxyindolea cetic acid	**Demin & Kolesnikova et al., 2021 [60]**

NDT -Novel Diving test, **LAD**-Light and dark test, (**PCUS)** -Prolonged chronic unpredictable stress,

Chemical stressor or stimuli

Chemical stressor responses include blood as an alarm material (which is known to cause panic reactions in a variety of fish species**) (Pfeiffer W. et al., 1977) [62]** Alarm pheromone exposure (15 min) produces behavioural alterations (like anxiety) in Zebrafish, detect alarm cues released by their injured skin cells through their olfactory system. **(Barreto R. et al., 2013) [63]** Benzo[a]pyrene is an environmental pollutant that increases the organic load in water, leading to overcrowding (OC) stress that is responsible for inducing behavioural alterations (like anxiety), Deficits in learning and memory are accompanied by histological alterations in the adult zebrafish brain. **(Aparna & Patri, et al., 2021) [64]** Continual exposure of bisphenol S (BPS) triggers oxidative stress, leading to abnormal anxiety and fear responses in adult zebrafish. Furthermore, it presents various adverse effects, such as cytotoxic, genotoxic, immune toxic, and repro toxic impacts in both humans and animals **(Salahinejad A. et al., 2021) [65].** Excessive levels of manganese (Mn), although an essential metal for living organisms, can be particularly toxic, especially for the central nervous system, and may induce anxiety in adult zebrafish. **(Ferreira, S. et al., 2022) [66]** Perfluorooctanoic acid induces aggression and anxiety-like behaviour in adult zebrafish by modulating both cholinergic and purinergic signalling biomarkers. **(Adedara et al., 2022) [67]** it seems like you've provided information about the impact of certain substances on the zebrafish brain, specifically aluminum (Al) and BPF (bisphenol F). Aluminum has been reported to modify oxidative stress, antioxidant defenses, metabolic alterations, and neurotransmission, resulting in anxiety in adult zebrafish (**Capriello et al., 2021) [68].** Additionally, chronic exposure to BPF has shown brain toxicity by dysregulation metabolic pathways in neurotransmitter systems and neurosteroid systems, affecting anxiety-related behaviors, as well as learning and memory function in adult zebrafish. **(Kim & Hwang et al., 2022) [69]** Oxy tetracycline (OTC) is an antibiotic, but it also shows an antigenic effect in zebrafish by affecting whole-body cortisol levels and the endocrine system**. (Gusso et al., 2021) [70]** Methyl mercury (MeHg) is a ubiquitous pollutant that causes developmental neurotoxicity (anxiety), even at low levels, in all life stages of zebrafish **(Glazer et al., 2021) [71].**

Table 2: Chemical reagent induces anxiety Zebrafish Model and observational parameter.

About chemical that induce anxiety	Pathology	Behaviour models	Biochemical	References
'm substance it s released from the skin of injured fish that causes an immediate alarm reaction among fish nearby, leading to the dispersal of a school formed by members of the same species. Alarm substance exposures (15 min) in zebrafish induce anxiety.	The induction of anxiety occurs through the activation of the HPI- axis center in fish.	LAD test,	cortisol	Abreu & Giacomini , et al., 2017 [80]
Benzo[a]pyrene is environmental pollutant, that increase organic loading water and leading to overcrowding (OC) stress that responsible for inducing behavioral alterations (like anxiety) learning and memory deficiency with histopathological changes in adult zebrafish brain. In order to cause OC stress (12 fish/L) in adult wild zebrafish exposed to B[a] P (0.2 mg/L−1) in water, acutely.	Numerous neurotransmitters, such as noradrenaline, acetylcholine, dopamine, and serotonin, are modulated by baP. The quantity of N-methyl-D-aspartate receptors (NMDARs) at the synapses is a key regulator of working memory, brain function, and cell survival.	NDT & LAD test, and T-maze test	Oxidative stress biomarkers like glutathione level catalase activity	Aparna & Patri, et al., 2021 [64]
Bisphenol S (BPS) Epoxy resins, personal care items, and many other products all contain the chemical bisphenol S (BPS). Chronic exposure to BPS and 17-estradiol (E2) at concentrations of 1, 10, and 30 g/L for 75 days causes oxidative stress, abnormal anxiety, and fear responses in adult zebrafish.	BPS-induced neurotoxicity and alterations in the brain's oxidative status results in impaired anxiety and fear responses in the brains of adult zebrafish perhaps through generating oxidative stress.	NDT test, Fear related test.	catalase, glutamine peroxidas, copper/zinc superoxide dismutase, and manganese superoxide dismutase antioxidant test	Salahinejad & Attaran, et al., 2021 [65]
Manganese (Mn) Metals like **manganese (Mn)** are necessary but excessive Mn can be harmful, particularly for the central nervous system, and can make adult zebrafish anxious. Fish were given doses of 0.5, 2.0, 7.5, and 15.0 mg/L of MnCl2 for 96 hours while being monitored for locomotor and anxiety-related behavioural characteristics.	Higher accumulations of Mn can increase AChE in the brain and increase whole-body cortisol, which is responsible for anxiety in adult zebrafish.	NDT & LAD test, and Social preference test,	Cortisol. AChE	Ferreira & Loreto, et al., 2022 [66]
PFCAs & FOA One of the **perfluoroalkyl carboxylic acids (PFCAs)**, perfluorooctanoic acid (PFOA) is utilized in both industrial and domestic settings for things like metal plating, medicines, cosmetics, surfactants, paper coatings, fire retardants, pesticides, and refrigerants. To look into the effects of PFOA (0, 0.25, 0.5, or 1.0 mg/L) administered for 7 days on adult zebrafish's aggressive and anxious behaviour.	By modifying both cholinergic and purinergic signalling indicators, PFOA causes anxiety. ADP hydrolysis was unaffected by PFOA, however ATP and AMP hydrolysis were dramatically boosted at the highest measured dose.	novet diving test, social preference test, and mirror-induced aggression test	Nucleotide hydrolysis assay AChE Puriner signal pathway	Adedara & Souzaet, et al., 2021 [67]
Aluminium (Al) is a harmful pollutant that poses a major risk to the environment and has been shown to be toxic to both terrestrial and aquatic	Al toxicity and its correlation with brain oxidative stress, changes in metabolism, and neurotransmission that are responsible for anxiety The	Light/Dark, social preference test, shoaling	superoxide dismutase, glutathione peroxidase and glutathione S-	Capriello, & Félix, et al., 2021

organisms. In adult zebrafish, aluminium exposure (11 mg/L at 10, 15, and 20 days) alters behaviour and induces oxidative stress in the brain.	activity of antioxidant enzymes increased after short-term exposures and tended to decrease or stabilize over longer periods.	behaviour and mirror test,	transferases, and the metallothioneins	
Bisphenol F (BPF) When exposed chronically to Bisphenol F (BPF) at environmentally relevant concentrations (0.001, 0.01, and 0.1 mg/L) for a duration of 4 weeks, BPF demonstrates neurotoxic effects by penetrating the blood-brain barrier and accumulating in brain tissues. This accumulation leads to anxiety-like behaviors in adult zebrafish.	The toxicity of BPF in the zebrafish brain led to the disruption of metabolic pathways associated with neurotransmitter and neurosteroid systems. RNA-seq analysis indicated that BPF exposure influenced various pathways, including metabolic pathways, CA-signaling pathways, neuroactive ligand-receptor interactions, and the gonadotropin-releasing hormone signaling pathway etc.	NDT & LAD test, T- maze learning and memory test locomotors test	Choline 17β-estradiol, cortisol, pregnenolone, and De hydro epiandrosterone in neurosteroid systems RNA-seq analysis	Kim & Hwang et al., [69]
Oxytetracycline (OTC) Fish farm infections are treated with Oxytetracycline (OTC). Additionally, it demonstrates anxiogenic effects on behavioural metrics and whole-body cortisol levels in zebrafish. We also note that OTC exposure (10, 20, and 100 mg/L) results in a phenotype that is similar to anxiogenesis in the novel tank test.	it's been observed that exposure to OTC (at concentrations of 10, 20, and 100 mg/L) induces an anxiogenic-like response in zebrafish, as evidenced by changes in behavioral patterns and whole-body cortisol levels.	Novel diving test social interaction test	whole body corisol test	Gusso, & Altenhofen et al., 2021 [70]
Methylmercury (MeHg) Even at low levels, the ubiquitous contaminant methyl mercury (MeHg) has been proven to have developmental neurotoxic effects. Throughout embryonic development.	zebrafish were exposed to sub-acute doses of MeHg (0, 5, 10, 15, 30 NM), and adult zebrafish were examined for anxiety-related behaviours and locomotor activity at all life stages, which increased anxiety-related responses.	Novel diving test, Light dark test locomotors test tap assays	cortisol	Glazer & Brennan, et al., 2021 [71]
High-fat diet (HFD) A high-fat diet given as food for two weeks induces obesity in adult zebrafish.**)**	HFD on CNS influences behaviors linked to anxiety-like responses, aggression, social preference, and memory, all crucial for survival and reproduction.	NDT & LAD test, Social preference test, Mirror test, Inhibitory avoidance test, morphological test.	Glucose, cholesterol, triglycerides and visceral adiposity	(Picolo & Quadros, et al., 2021)[52].

NDT -Novel Diving test, **LAD**-Light and dark test,

Physical/Mechanical and chemical stressor response on cortisol

The primary neurotransmitter that controls the release of adrenocorticotropic hormone (ACTH) from the pituitary, subsequently triggering the release of glucocorticoids (like cortisol) from the adrenal gland, is corticotrophin-releasing factor. This factor is stimulated by stress and originates from the hypothalamus. In teleost fish and zebrafish, the HP-interrenal axis functions as the HPA axis homolog. **(Wendelaar Bonga, 1997)[72]** When zebrafish encounter stress, the synthesis and release of glucocorticoids occur, similar to the production of cortisol in humans and corticosterone in rodents. These stress-induced hormones reach the target organs in the organism. **(Jo¨els M. & Karst et al., 2018) [73].** these stress hormones' biological actions are mediated by receptors called mineralocorticoid (MR) and glucocorticoid (GR) receptors. **(Katsu Y& Baker, et al., 2021)[74].** additionally, glucocorticoids are in charge of biofeedback control of hypothalamic and CRH and ACTH release. **(De Kloet E & Joëls, M et al., 2005).[75]** When fish exposed to physical stresses like chasing or spatial confinement and chemical stressors like alarm material or blood showed a rise in cortisol levels, **(Abreu & Giacomini et al., 2017) [76**] Although zebrafish that have been acutely exposed to a modest stressor, such as a 1-min air exposure, exhibit unaltered brain gene expression for interleukins (IL) IL-1 and IL-6, brain-derived neurotropic factor (BDNF), interferon gamma (IFN-ý), and tumor necrosis factor-alpha (TNF-α), **(Kirsten & Pompermaier et al., 2020),[77]** All of these genes are unregulated in the brain under more extreme acute stress conditions, such as a 90-min exposure to cold water, bright light, vortex, shallow water, and constraint. **(Yang & Wang et al., 2020)[78].** Acute exposure to both physical and chemical stressors raises cortisol levels throughout the body and induces anxiety. **(Abreu & Giacomini et al., 2017)[76].**

Table 3: Summary of zebrafish cortisol responses to various stimuli

Type of stress	Methodology	Pathology	Species	Cortisol level	References
Chemical stressor	Exposure to the alarm substance of conspecifics (15 min) in zebrafish	Induce anxiety throughout the hypothalamus-pituitary-interrenal axis (HPI) centre in fish.	zebrafish	Increase in whole body	Abreu & Giacomini, et al., 2017 [76]
Physical stressor	Physical restraint (15 min)	Fish's hypothalamus-pituitary-interrenal axis (HPI) is impacted.	zebrafish	Increase in whole body	Abreu & Koakoski et al., 2014 [54]
	Net chasing (for 1 min)	Stressors impact HPI-endocrine function.	Jundi´a, nile tilapia.	Increase in plasma	Cericato & Neto, et al., 2008 [91]
	Chasing with a net (duration 2 min)	In fish, physical stress affects the HPI centre.	zebrafish	Increase in whole body	Abreu & Koakoski et al., 2014 [54]
	Social isolation for 15 min	The HPI centre in fish is the target of physical stress.	zebrafish	Whole body	Shams, & Seguin et al., 2014 [93]
	Beaker stressor	Fish's HPI centre is impacted by physical stress.	zebrafish		kalueff & Stewart, et al., 2014[06]
	Changes in the lighting schedule, plus net chasing, net restraint, air-exposure, crowding, water level decrease, and isolation.	Fish's HPI centre is targeted by physical stress.	zebrafish	increase cortisol	Pavlidis & Theodoridi et al., 2015 [92]
	Air exposure (1 min)	work on HPI axis	zebrafish	Whole body	Abreu & Koakoski

					et al., 2014 [54]

Behavioral models used in Anxiety

In anxiety behavioral models for mammals, where species-specific defensive behaviors represent fear, anxiety, and stress, the endophenotypes method is easily applied. (**Hasler & Drevets, et al., 2004) [79]** Various behavioral models, including the open field, novel tank diving, light and dark tests, and shoaling, are essential for observing different disease types. Anxiety-like behavior assessment typically involves open-field exploration, often performed in a circular open tank or arena. Animals are placed in a new tank, and their exploratory actions, like thigmotaxis, exploratory swimming, freezing spells, and erratic movements, are recorded. **(Cachat & Canavello et al., 2011) [80].** the novel tank diving test has been a key focus in zebrafish anxiety research, especially in exploring drug interventions. This test observes that in a new tank, zebrafish initially tend to spend about 70–85% of the first minute in the bottom third. However, over a 5- or 10-minute period, this preference decreases to around chance levels. **(Gerlai & Fernandes et al., 2009) [81]** Research on fear and anxiety frequently uses novelty-based tests, such as the light/dark test, in behavioural neuroscience. To learn more about behaviour, better identify, and draw attention to changes in the brain and behaviour, behavioural models are utilized **(Miller & Gerlai et al., 2007) [82].** Zebrafish exhibit natural shoaling behavior. The shoaling test involves observing a test fish placed in the center of a tank, with two compartments beside it—either empty or occupied by other fish. This approach helps reveal shoaling preferences and the general inclination of the test subject to shoal. However, it doesn't allow investigation into the internal dynamics of free-swimming shoals. Analyzing the dynamic behavior of fish in a moving shoal is needed for a comprehensive understanding. **(Mansur & Dos et al., 2014) [83]**

Behavioral observational models

Model Name	Observation	Use	Reference
Open-field	Avoidance behavior in zebrafish can be assessed by observing the time spent in the center field and the inclination towards thigmotaxis. For monitoring activity levels, the total distance traveled by the fish can be measured. Additional behavioral patterns like burst swimming, freezing, erratic swimming, and thigmotaxis can also be considered for a comprehensive assessment.	Anxiety depression bipolar pain	Gerlai & Fernandes et al., 2000 [81]
Novel tank diving test	Avoidance behaviors in zebrafish can be evaluated by measuring the time spent in the bottom portion of the tank, as well as the number and latency of transitions to the upper half. To monitor activity levels, the total path length traveled by the fish can be measured. Other behaviors such as burst swimming, thigmotaxis, freezing, and erratic swimming can also be observed for a more comprehensive assessment.	Anxiety Depression locomotors test	Gerlai & Fernandes, et al., 2009 [81]
	Avoidance: Time spent in light area Activity: Activity in dark area; total entries Arousal: Blood pressure; heart rate; number of fecal boil; number of rearing	Anxiety Depression Autism	Mansur, & Dos et al., 2014 [83]
Shoaling	For examining avoidance behaviors, observing the average distance among shoal mates within the tank can provide insights into social interactions. In assessing activity levels, tracking the	Psychiatric disease	Miller & Gerlai, et al., 2007 [82]

	total path length specifically for the focal fish is crucial. There weren't any other specific measures noted in this context.		

Standard drug use in treatment of Anxiety

The medications used to treat panic Anxiety attack disorder (PADs), generalized anxiety disorder (GADs), social anxiety disorder (SADs), and specific phobias (SPs) are discussed in this review. These medications include selective serotonin reuptake inhibitors (SSRIs),**(Bakker & Van et al., 2002; He H, & Xiang Y, et al., 2021)[85-86]** serotonin norepinephrine reuptake inhibitors (SNRIs), Tricyclic Antidepressants (TCA), Monoamine Oxidase Inhibitors (MAOI) Benzodiazepines (Clonazepam) azapirones (such as buspirone), antipsychotics, antihistamine Obsessive-compulsive disorder and posttraumatic stress disorder are not included in this review. **(Mula & Pini et al., 2007; Balon & Starcevic et al., 2020; Guaiana & Barbui et al., 2010; Chessick & Allen et al., 2006)[86-90].**

Current treatments for anxiety disorders

Class	Drug	Mechanism of action	FDA approvals	Off-label uses	Reference
SSRI	Fluoxetine Sertraline	Work through Selective 5-HT reuptake inhibitor	PADs PADs, SADs	GADs, SADs GADs	Bakker A. & Van Balkom et al., 2002 [85]
SNRIs	Duloxetine Venlafaxine	Act on 5-HT, NE (and DA) reuptake inhibitor	GADs GADs	PADs, SADs,	He H, & Xiang Y, et al., 2021 [86]
TCAs	Clomipramine Imipramine	These work on NE and 5-HT reuptake inhibitor	None	GADs, PADs, both	Bakker A. & Van Balkom et al., 2002 [85]
MAOIs	Phenelzine	Directly act on MAO inhibitor	None	GADs, PADs, SADs	Bakker A. & Van Balkom et al., 2002 [85]
GABAergic drugs	Pregabalin Gabapentin	Unclear, may modulate Ca channels	None	GADs, PADs, SADs.	Mula & Pini et al., 2007 [87]
Benzodiazepines	Clonazepam Alprazolam	work through GABA-A agonist	PADs Anxiety, PADs	Anxiety, GADs, PADs, SADs	Balon & Starcevic et al., 2020 [88]
Antipsychotics	Olanzapine	Act on different receptor like- D_2, 5-HT_2 H_1 antagonist	None	GADa, PAD, SADs,	Balon R. & Starcevic V. et al., 2020 [88]
Antihistamines	Hydroxyzine	H_1 antagonist	anxiety,	anxiety, PADs, SADs	Guaiana & Barbui et al., 2010 [89]
Other anxiolytics	Buspirone	Mainly act through 5-HT_{1A} partial agonist	anxiety,	GADs,	Chessick & Allen et al., 2006 [90]

Key: 5-HT-Serotonin; DA-Dopamine; D2-dopamine-2 receptor; FDA-Food and Drug Administration; GADs-Generalized Anxiety Disorder; GABA-Gamma Aminobutyric Acid; H1-Histamine 1 receptor; MAO-Monoamine Oxidase; MAOI-Monoamine Oxidase Inhibitors; NE-Norepinephrine; PADs-Panic Disorder; SSRI-Selective

Serotonin Reuptake Inhibitor; SNRI-Serotonin Norepinephrine Reuptake Inhibitor; SAD-Social Anxiety Disorder; TCA-Tricyclic Antidepressants.

Future prospective

Identification of effective anxiety-inducing methods for zebrafish is pivotal for advancing behavioral studies and pharmaceutical interventions. This comprehensive review, highlighting various methods both behavioral and biochemical for inducing anxiety, presents an opportunity to refine zebrafish anxiety models, paving the way for more nuanced behavioral studies and effective pharmaceutical interventions.

The review underscores the need for further investigations to enhance our understanding of zebrafish anxiety models. By identifying gaps in knowledge and providing a collective summary of anxiety-inducing methods, it acts as a catalyst for future research, encouraging explorations into uncharted territories and deeper investigations into refined anxiety-inducing methodologies.

The findings present potential translational applications in studying anxiety-related disorders in humans. The similarities between the anxiety-inducing methods and daily life stressors experienced by humans offer opportunities for utilizing zebrafish as a model to explore anxiety-related disorders in humans, potentially leading to more effective interventions and treatments."

Furthermore, the reviewed methods suggest avenues for the exploration of novel methodologies and technologies to enhance or innovate anxiety induction in zebrafish models. This direction could facilitate the development of more sophisticated methodologies, aiding in refining and enhancing the zebrafish anxiety model.

Conclusion

In conclusion, this review article has provided valuable insights into the complex landscape of inducing anxiety in zebrafish. It has addressed the challenges researchers encounter in selecting appropriate methods for anxiety induction, ultimately striving to consolidate these methodologies. Mechanical and chemical stressors were at the forefront of the discussion, each possessing unique characteristics and implications. The study highlighted the difficulties in choosing the most suitable method for inducing anxiety, emphasizing the importance of consolidating and comparing these diverse approaches. Mechanical stressors predominantly acted on the hypothalamic-pituitary-interrenal (HPI) axis, unraveling the underlying stress-inducing mechanisms. In contrast, chemical inducers exhibited versatility in their modes of action, complicating the selection process. Pathophysiological changes associated with anxiety induction were linked to the activation of the HPI axis, with cortisol serving as a consistent marker of these alterations. This insight provided a deeper understanding of the physiological responses in zebrafish under anxiety-inducing conditions. The review also delved into a cost-effectiveness analysis, shedding light on the financial implications of employing mechanical versus chemical stressors. While mechanical methods might necessitate more extensive setups and time, chemical inducers could prove more costly, reflecting the multifaceted nature of their actions. Despite the comprehensive exploration, a clear advantage for any specific anxiety-inducing method in terms of efficiency or cost-effectiveness did not emerge. Instead, each method demonstrated its unique attributes, making it challenging to declare a single modality as superior. This highlights the need for continued research to refine our understanding of zebrafish anxiety models and their applications in various experimental contexts.

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CONFLICT OF INTEREST

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