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# A Review on Zebra Fish as a Preclinical Model for Natural Drug Discovery

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**Abstract:** Natural products represent a significant reservoir of unexplored chemical diversity for early-stage drug discovery. Zebra fish has been used as a preclinical model for identifications of various natural compounds presents on natural products, early life stage tests with zebra fish used to detect toxicity of compounds from natural bloom extract on their embryolarval development. The use of Medicinal plants for treatment of neurological & neuro-degenerative disorders, cardiovascular and anti-melogenesis are very common. Bitter gourd shows anti-diabetic effects by using zebra fish model in a designed experiment. Zebra fish has been used as a tool for discovering of phyto-chemical compounds in natural products and their activities such as, fruit of *Terminalia chebula* possessing the activity of anti- epileptic, *Oxygonum sinuatum* and *Plectranthus barbatus* showed anti-angogenesis with the active compounds Emodin & Coleon A lactone. Cardiac toxicity by the fruit extract of *M. charantia* reported. Zebra fish is gaining more interest as a preclinical tool for drug discovery because it has been demonstrate that pharmacokinetic analysis can be under-taken in this model. The study concludes that and gives a desktop reference to the new researchers in finding out the earlier attempts which were made on zebra fish (*Danio rerio*) as a preclinical tool in natural product discovery.

**Keywords:** Zebra Fish, Natural Products, Diabetes Mellitus (DM), Hypertension, Neurological Disorders, Bitter Gourd

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## 1. Introduction

Zebra fish play an important role as a preclinical tool in the research and to understand many diseases, its preferable over the mammals because of its advantages that have high genetic homology with human beings, similarity of organs as well as tissues, its fast extrauterine development and growth [1], transparent embryos which aid to direct observe its organs system [2], as well as very short reproductive cycle, relatively small size about (1-5 nm), also possibility to produce and more numbers of embryos and larvae because of its high fecundity and fertility relatively low cost compared with mammals [3], screening of herbal products and medicines even with small quantities, less and controllable duration of testing, suitable for large scale drugs screening. zebra fish is used as a model for the treatment of several diseases conditions such as, diabetes mellitus (DM) type 2 showing expression of pck1 gene which play important role in control of gluconeogenesis. also it is a model in antihypertensive activity due to possibility of expression of endothelial. anti anxiety model of zebra fish

shows conserved responses [4]. also in treatment of angiogenesis due to the inhibition of intersegmental vessels gene, anti-melanogenic activity on the melanin pigmentation process can easily observed on the surface of zebra fish [5], also zebra fish model in neurodegenerative disorders such as Alzheimer, Parkinson, schizophrenia and Huntington diseases shows positive results. *Danio rerio* model also for anti cancer activity and targeting as well as treatment of cancer cells, in addition to analgesic and anti-inflammatory model. zebra fish provides preclinical and biological model for the identification as well as discovery of natural products And its its different activities via the bioassay guided isolation of plants products such as pharbitis nil seeds part have antiepileptic effect, tephrosia purpurea leaves part possess inhibitory action on antiacetylcholinestrase, also whole plant of *Dysosma vesipellis* possessing angiogenesis activity on danio rerio model model [6]. A model of zebra fish embryo for the study of high blood pressure and screening of antihypertensive medicinal plants specially Iranian plants such as of zebra allium hirtifolium was successfully performed [4]. zebra fish possess blood brain

barrier that is structurally as well as physiologically and pharmacologically similar to that of the humans beings, so it has been used for in vivo evaluation and assessment of natural products delivering and permeation via blood –brain barrier and helping in the treatment of various central nervous system disorders and it is also has validated for central nervous system drug screening [7]. In toxicological testing part also zebra fish (*Danio rerio*) is an excellent model for the developmental toxicity screening and detection for the natural products and their extracts, such as the pant bitter gurd (*Momordica charantia*) seeds and fruits parts shows teratogenic effect on fetus of zebra fish embryo [8]. A part from the toxicity screening model of zebra fish on plant material also model for the treatment of pigmentation on the skin or can called it melanogenesis successfully treated by using rhizome part of the plant *Ligusticum sinense* extract [5]. the leaf of *p.malayana* possess antidiabetics activity and induced type 1 diabetes mellitus on zebra fish model (*Danio rerio*) [9]. zebra fish is potential model for the drug discovery, development of treatment pathways, as well as drug screening for a wide range of natural products either plants and its extracts also for the micro-organisms (eg: fungi & bacteria) [10].

## 2. Zebra Fish Model in Diabetes Mellitus (DM)

Diabetes mellitus is a metabolic disorder that due to destruction of pancreas and disability to produce insulin known as type 1 DM, or resistance of insulin towards the receptors called as type 2 DM. Many of the bioactive natural products that derived from the herbal medicines have been perfect sources of a new drugs for number of diseases. Numerous of natural products that derived from medicinal plants extracts have been shown successful and potential effect as anti-diabetics agents in cell based screening. Zebra fish (*Danio rerio*) have been reported as a perfect model for the screening of natural products that have anti-diabetics activity, its provides excellent experimental model system for fast confirmation the therapeutic effects of a new anti-diabetic agents [2]. The aqueous extract of *Psychotria malayana* leaf part successfully showed anti-diabetic activity on the developed zebra fish model, a guideline have been developed and also used in the process of evaluation of the anti-hyperglycemic activity of *Psychotria malayana* leaf part extract, the anti-diabetic activity for the plant leaf extract *psychotria malayana* have been evaluated by perform the analysis of blood glucose sugar levels as well as liquid chromatography – mass spectroscopy fingerprinting technique to zebra fish model, so after the evaluation *p.malayana* leaf plant extract completed can understand the following, that the plant extract of *p.malayana* at the quantities of (1, 2, 3 g/kg) has been successfully lower the blood glucose levels in the zebra fish model for diabetes mellitus. additionally to that the liquid chromatography – mass spectroscopy based fingerprinting technique have been indicate that the quantity of 3 g/kg of the plant extract have more effectiveness comparing

with the other doses [9]. various medicinal plants and plants extract shows the anti-diabetic activity on the zebra fish model such as the Iranian medicinal plants that have been used for several years in the treatment of several diseases specially diabetes mellitus, examples of medicinal plants that traditionally used in Iranian medicine as anti-diabetic agents such as *Doctyorchiza iberica* plant tubers part, *Achillea wilhelmsii* the flower part, leaf and steam part of *cichorium intybus*, *Berberis integeriam* leaf and flower parts and *Tanacetum polycephalum* leaves. all this medicinal plants have been successfully studied and evaluated for antidiabetic activity by Iranian scientists, additionally medicinal plants also like *Utrica dioica* leaf and steam parts and *Spiranthes spiralis* tubers have been successfully determined the active composition responsible for the anti-diabetic activity, on the other hand some of the medicinal plants such as *Hausknechtia elymaitica* as well as *Gymnadenia* still there is no confirmation studies or evidence on their activity and effect on the diabetes mellitus, so zebra fish embryo is the perfect model for the screening and find out more information as well as knowledge about the plants extracts and its different activities also provide better evaluation to the natural products as a preclinical drug discovery model [4].

## 3. Zebra Fish Model in Angiogenesis

Angiogenesis can be defined as the process of formation of a new blood vessels from the pre-existing vascular system via endothelial. angiogenesis can be caused due to different factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), Angiogenesis is starting point to development of several diseases such as cancer, psoriasis, rheumatoid arthritis, retinopathy and endometriosis. There are several natural products and plants extraction identified that have antiangiogenesis activity on zebra fish model. screening of natural products and plants extracts has been more important to find out more effective as well as safe angiogenesis inhibitors. The antiangiogenesis activity of the following natural products such as genistein, Camptothecin, kaempferol, ferulic acid and quercetin have been studied and evaluate. Using in vivo zebra fish embryos model a natural product mundoserone have been identified that contain certain antiangiogenesis activity and found out that the mundoserone directly inhibits inter segmental vessels (ISV) formation in zebra fish embryos model additionally it is also acts by the reducing the expression of slit guidance ligand 3 (SLIT3), its also can be act on roundabout guidance receptor 1 (ROBO1) and also 2 fibroblast growth factor receptor (FGFR). Its also acts by increasing the expression of NOTCH1AV. Mundoserone is an important angiogenesis inhibitor that may also act by up-regulation of the SLIT/ROBO1 and FGFR/PTP-RB, as well as up regulation of NOTCH1A signals pathway [11]. On the other hand a Bioassay guided zebra fish for isolation of angiogenesis inhibitors from the east African medicinal plants also examples for these plants, such *oxygonum sinuatum* and *Plectranthus barbatus* extract have been successfully studied and evaluated for the antiangiogenesis activity on zebra fish model. that acts

by inhibits the vascular development, danio rerio guided have been identified the the various compounds of these plants extract as emodin, and its acts by inhibits the particular protein kinase CK2 as well as coleon A lactone. these tow compounds emodin and coleon A lactone will inhibited mammalian endothelial cell proliferation, migration and also the formation of tube in vitro [3]. plants products and extracats have antiangiogenesis activity such as *Rehmannia glutinosa* root part the molecule responsible for the activity is norviburtinal, the whole plant of *Rhynchosia glutinosa* the molecule responsible for its activity was identified as Rhynchoviscin and *Dysosma versipelli* also have the antiangiogenesis effect the molecule is kaempferol [6].

#### 4. Zebra Fish Model in Neurological & Neuro-degenerative Disorders

Neurodegenerative disorders is a dangerous condition where the damage to the brain takes place and disturbance of brain function occurs. neuro-degenerative disorder is a group of disorders that occurs due to neurotransmitter dysfunction, includes Alzheimer disease, schizophrenia, huntingtons disease and Parkinson disease. Zebra fish have been examined as well as reported as successful animal model for the screening and evaluation of natural products used in neurodegenerative disorders. The Natural products are small in size molecules derived from various living organisms that can be plants, bacteria and fungi, this natural products was reported as secondary metabolite. zebra fish provides an excellent biological model for the identification of natural products derived drugs through the in vivo bioassay-guided chromatographic fractions and that required a little quantities of each components. For the bioassay guided separation of natural products each of the chromatographic fraction part is to be undertakes for biological evaluation to select the active fraction only [6]. There is various factors that causes Alzheimer disease may be the accumulation of intracellular amyloid beta as well as intracellular hyper phosphorylated tau tangles these factor lead to memory loss [12]. Zebra fish model in Alzheimer disease have many genes similarity with the human beings genes that play very important role in Alzheimer disease because of this similarity zebra fish identified as perfect model for Alzheimer disease. Zebra fish genes *pseol* and *psen2* identified as human *PSEN1* and *PSEN2* orthologs. Also the genes *appa* and *appb* are *APP* co-orthologs in human. Zebra fish model in Parkinson disease, approximately about 6 genes is linked with Parkinson disease have been identified its zebra fish is a perfect model for Parkinson disease, these genes are *parkin*, *DJ-1*, *PINK1*, alpha synuclein, *UCHL-1* and *LRRK2* [6]. There is various plants shown neurological activity in zebra fish model, plant with anti-epileptic activity examples *Terminalia chebula* fruit part, *Anacyclus pyrethrum* root part, *Pimpinella anisum* seeds, *Laurus nobilis* leaf and *Nigella sativa* seeds as well as fruit parts. Plants with anti-anxiety activity identified in zebra fish model and the responsible compounds for the activity

have been reported for the following plants, *Echium amoenum* the flowers part, aerial part of *Crocus sativus* and *Glavcium spp* leaves [4]. The traditional plant extract of *Coriandromsativum* successfully identified in zebra fish model that induce anti anxiety effect and associated with sedative effective also [1].

#### 5. Zebra Fish Model in Toxicity

Zebra fish (*Danio rerio*) have been successfully identified as perfect organism for toxicity model used for the identification as well as screening of the different natural products toxicity. The toxicity of the plant extract of *Momordica charantia* have been studied and analyzed on zebra fish model, the seed of *M.charantia* extract showed toxicological effect on zebra fish embryos fetus. The major merits of zebra fish embryos that its develops outside of mother and this how the effect on mother can be avoided on fetus development, so the toxicological effects of any compounds and by easily identified on the zebra fish embryos. The seed extract of the plant *Momordica charantia* when its has been tested for its toxicity effects its showed very different levels of toxicity in zebra fish embryos a part from that have been identified that it produce teratogenicity like effect in the developing embryos. At the sub – LC<sub>50</sub> dose they found out that seed extract of *m.charantia* inhibits the growth and development of zebra fish embryos. Different doses of the seed extract of *m.charantia* have been given to zebra fish embryos and its shown abnormalities in the growth in dose dependent manner. Embryos which have been given 30 microgram/ml of the seed extract of the drug have been shown more inhibitory effect on the growth, and another treated with 20 microgram/ml of the seed extract of *m.charantia* produced cardiac hypertrophy along with cardiac edema. The zebra fish embryos which have been treated with 15 microgram/ml of seed extract shown a mild levels of growth arrest in addition to sever cardiac toxicity, the zebra fish embryos which is given 10 microgram/ml of the seed extract showed very sever cardiac hyper atrophy as well as cardiac edema. finally the treated embryos of 5 microgram/ml by the seed extract of *m.charantia* have showed mild condition of developmental arrests and also sever cardiac hypertrophy. Gas chromatography – mass spectroscopy has been used and identified the active chemically constituents of *m.charantia* includes, *1,2-cyclopentanedione*, *Elemol*, *Beta-eudesmol*. The teratogenicity of seed extract of *m.charantia* should be avoided or care use for the prevention of fetus damage embryos [8]. Zebra fish at there beginning stages of life has been used for the detection of the toxic effect of plant extract, such as *Microcystis aeruginosa* natural bloom extract used to identify the toxicity on the developmental stages of zebra fish embryolavral. When zebra fish treated with the extract of *M.aeruginosa* showed malformation and observed edema, and curving in tail [13].

#### 6. Zebra Fish Model in Melanogenesis

Melanogenesis is condition where there is over production of

melanin. melanin is a brown black pigment that its main function is color of skin, hair and eyes. its also products the skin from the effect of ultraviolet radiation and the environmental pollutants. the over melanogenesis can leads to changes is skin color like darkening and several abnormalities in hyper pigmentation. Zebra fish as a model in melongenesis, that have been successfully identified by the Chinese medicinal plant called as *Ligusticum sinense* rihzoma part in olden days it has been use as cosmetic for the whitening of skin body and hydrating of skin. Assay guided purification using semi-preparative high performance liquid chromatography with the

spectroscopic analysis have been performed for the purpose of determine and identify the active compounds present in *L.sinense* [5], that is (*1. Lignin 2.cis-4-pentylclohex-3-ene-1,2-diol*) responsible of the anti-melanogenesis activity [6]. The investigation showed that chemical constituents of *L.sinense* having significant anti-pigmentaion effect on zebra fish embryos. The pigmentation process that present on the surface of zebra fish embryos easily for observation that made zebra fish particular useful model for in vivo investigation for melanogenic inhibitors or stimulators [5].

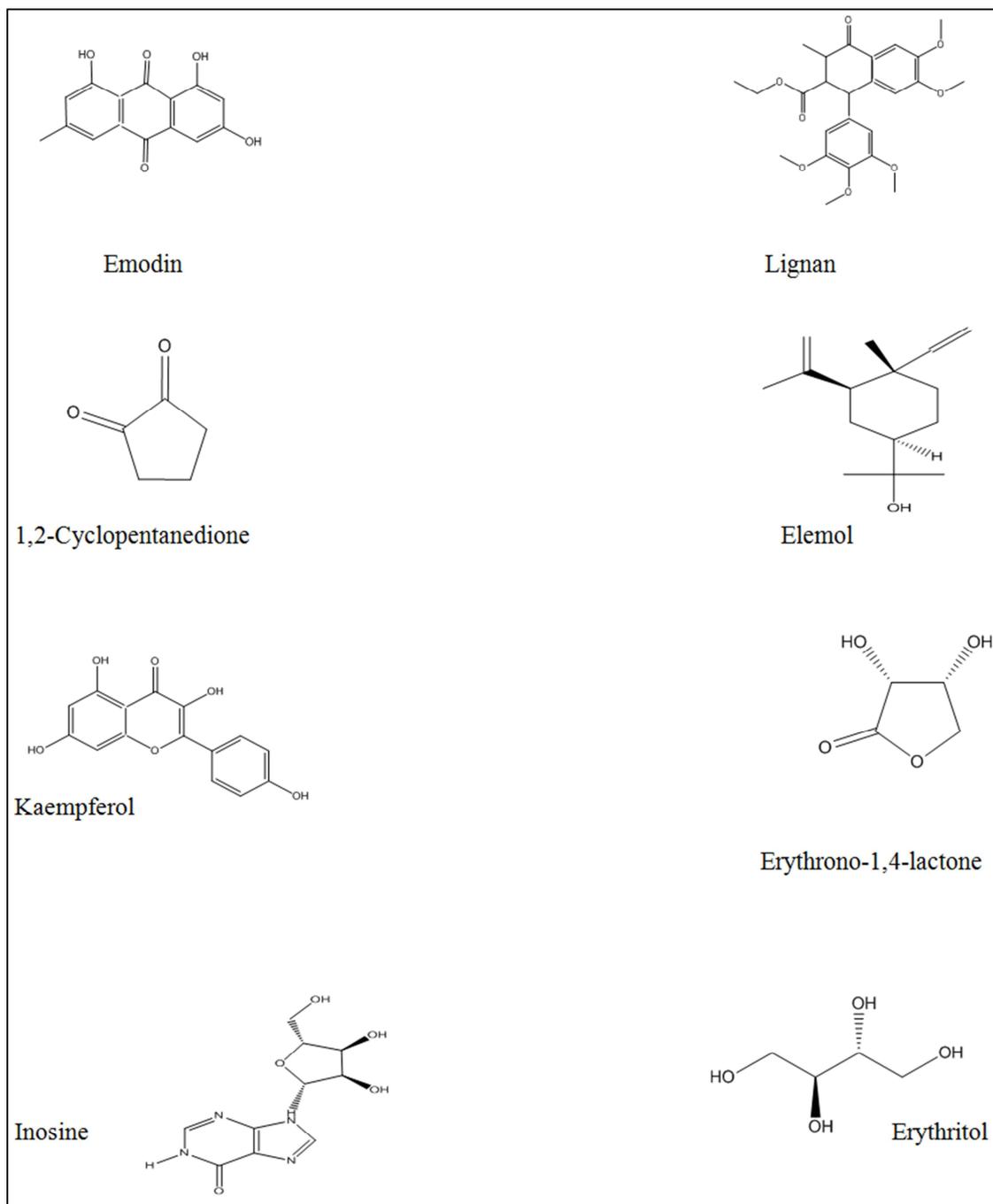


Figure 1. Chemical structures of compounds discussed in this review.

**Table 1.** Zebrafish bioassay-guided isolation and structurally characterized natural products.

| Zebra fish model               | Plant sources  | Active principle  | Reference |
|--------------------------------|--|---|-----------|
| Acetylcholinesterase inhibitor | <i>Tephrosia purpurea</i>                            | Trans – tephrostachin   | [6]       |
| Anti-melanogenesis             | <i>Ligusticum sinense</i> (rhizome)                  | Lignin, cis-4-pentylcyclohex-3-ene-1,2-diol.  | [5, 6]    |
| Toxicity model                 | <i>Momordica charantia</i> (seed, fruit)             | 1,2-cyclopentanedione, Elemol, Beta –udesmol.   | [8]       |
| Toxicity model                 | <i>Microcystis aeruginosa</i>                        | Microcystins  | [13]      |
| Anti convulsant                | <i>Solanum torvum</i> (leaf)                         | Paniculonin A, Paniculonin B  | [6]       |
| Anti-anxiety                   | <i>Coriandrum sativum</i>                            | <i>Coriandrum sativum</i> extract (CSE)   | [1]       |
| Anti-angiogenesis              | <i>Dysosma versipellis</i>                           | kaempferol  | [6]       |
| Toxicity                       | <i>Nux vomica</i>                                    | <i>Nux vomica</i> extract   | [14]      |
| Angiogenesisinhibitors         | <i>Oxygonumsinuatum</i> <i>Plectranthus barbatus</i> | EmodinColeon A lactone  | [3]       |
| Anti-diabetic                  | <i>Psychotria malayana</i>                           | <i>p.malayana</i> extract tentative compounds,<br>Erythrono-1,4-lactone, Erythriol, Xylonic acid. | [9]       |
| Anti hypertension              | <i>Allium hirtifolium</i> (bulbs)                    | <i>A.hirtifolium</i> extract  | [4]       |
| Anti-seizure                   | <i>Sekletonema marinoi</i>                           | Inosine   | [6]       |
| Toxicity                       | <i>Acmella oleracea</i> (flowers)                    | <i>A.oleracea</i> extract   | [15]      |
| Anti angiogenesis              | <i>Rhynchosia viscosa</i> (whole plant)              | <i>Rhynchoviscin</i>  | [6]       |
| Anti epileptic                 | <i>Terminalia chebula</i> (fruit)                    | <i>T.chebula</i> extract  | [4]       |
| Anti- seizure                  | <i>Pharbitis nil</i> (seeds)                         | <i>Pharbitin</i>  | [6]       |

## 7. Conclusions

In summary, several observational studies have shown a connection between zebra fish and human neurological and cardio-vascular disorders. Zebra fish are proving to be an ideal model for screening as well as identifications of natural products and their activities in the treatment in different diseases as discussed in this review. The zebra fish is gaining more interest as a preclinical tool for drug discovery because it has been demonstrate that pharmacokinetic analysis can be under-taken in this model. For CNS drug discovery, zebra fish could be used as modules of CNS disease at the same time. Parkinson and Alzheimer diseases had already been well validated for CNS drug screening.

## Conflict of Interest

The author declare no conflict of interest.

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