

## Zebra Fish Animal Model on Tuberculosis: a Boon for Researchers Involved in New Drug Discovery



### Medical Science

**KEYWORDS:** Mycobacterium marinum, Zebra fish animal model, Mycobacterium tuberculosis.

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### ABSTRACT

*Tuberculosis have plagued mankind for so long a period in the history than any other microbe. With the emergence of drug resistant strains of Mycobacterium tuberculosis like MDR and XDR it's the right time to venture into new drug discovery. As the Mycobacterium goes for adaptive immunity against the synthetic chemical compounds given as drug and this habit is not seen against herbal drugs we need to focus on our ancestral wisdom to rule out the possible herbal formulations or drugs against this weird killer disease. This review explains about the emerging trend in using Mycobacterium marinum infected Zebra fish as a surrogate for the Mycobacterium tuberculosis disease model.*

### Introduction

Across the history of mankind, Tuberculosis has plagued and killed more people than any other microbial pathogen. Diagnosis of the disease in modern medicine became possible only after Robert Koch discovered Mycobacterium tuberculosis in the year 1882. Proper treatment protocols emerged only by the middle of 20<sup>th</sup> century. Indian system of medicine treated this disease from 5000 BC through herbal drugs(1). Ayurvedha named it as Rajayakshma while Siddha medicine called it as Shayarokam. Hippocrates identified tuberculosis as "Phthithis" in 460 BC and reported it to be the most widespread disease of that time(2). Prehistoric human skeletal(4000BC) remains and Egyptian mummies(3000-2400 BC) has showed evidences of tuberculosis(3).

### Tuberculosis Disease burden.

WHO in the year 2014 reported 9.6 million of new TB cases, 1.5 million deaths with 1.2 million among people who were HIV positive and 3,90,000 deaths among people who were HIV negative(4). South east Asia and Western Pacific regions collectively contributed to 58% of the Worlds TB cases in 2014. Next to African region, India has the largest number of cases(23% of the global total).

### Current status on drug discovery

Recently WHO announced the integration of traditional medicine into the National Health Systems globally, which becomes the best health care model for India to build a safe, affordable and effective health system as we are rich both in medicinal plant resources and traditional medicinal knowledge. It's the right time to invest in holistic health models rather than the existing interventional and iatrogenic models on health delivery. A large number of plant extracts examined against tuberculosis have shown significant activity. Cross resistance against a purified chemical compound is identified to be the cause for drug resistance in case of tuberculosis. Mycobacteria slowly adapts and develop resistance. An effective alternative could be a herbal drug.

In India, Herbs were used as medicine to cure diseases since the time of Rigveda. There were more than 80 herbs tried against mycobacterium tuberculosis with significant results against the same. For example, *Solanum trilobatum*, *Oldenlandia umbellata*, *Adhatoda vasica*, *Aegle marmelos*, *Carica papaya*, *Murraya Koenigii*(5)etc. With the tuberculosis menace still in the hit list and with the emerging drug resistant strains (so called superbugs) like MDR and XDR Mycobacterium, Scientists across the world is in hunt for antimicrobial drugs. As mentioned earlier, herbal based drugs are found to be safe, cost effective and reliable that prevents drug resistance. What we come across in such research process is the stringent clinical trial rules and the animal ethics rules governing the small animal research on diseases. And very important is the cost of the facility that can handle

drug discovery on tuberculosis.

### Zebra fish as an animal model

The recent emergence of the Zebra fish (*Danio rerio*) a tropical freshwater fish as an alternative animal model for drug research. George Streisinger and his colleagues in the University of Oregon established the methods to genetic manipulation of the species. National Institute of Health (USA) has considered it as the Third most important experimental organism for research. There is an increasing trend in the use of Zebra fish as a disease model in US and European countries although its less familiar in India. Although mostly its used in the fields of molecular biology, developmental biology, neurobiology and genetics research, it is gaining importance in the fields of cancer research, neurophysiology and drug discovery. The main reason behind this increase is the cost effective lab set up, easy breeding, see through transparent embryos, and less maintenance as the fish itself is a species that belongs to India. It breeds well in captivity with our Indian room temperature settings and the fries are born in 4days after the eggs are laid by the parents who spawn when kept in a dark room overnight. Simple is the procedure and it is amazing to watch these hatch in a small ordinary aquarium glass tank. Every institute can have a common breeding unit that caters to the need of individual researchers needs. Research methods that are standardized or custom designed can be made and the results can be obtained in a fortnight. One of the important point to note is that this animal model crosses the CPCSEA norms easily and hence there will not be much from those authorities, although Institutional policies can be made for breeding, handling, infection control and carcass disposing issues.

### Zebra fish animal model on tuberculosis

Mycobacteriosis has long been recognized as a source of morbidity and mortality in fish aquaculture(6). It was first reported in carp(*Cyprinus carpio*) from water contaminated with mycobacterium tuberculosis(7). Three mycobacterium species has dominated the fish diseases, *Mycobacterium marinum*, *Mycobacterium fortuitum*, and *Mycobacterium chelonae*. Out of this *Mycobacterium marinum* is the one which infects and causes disease in the Zebra fish that exhibits skin lesions and ulcers. *Mycobacterium marinum* can cause skin disease in humans when they come into contact with the infected fish as in keeping and cleaning aquariums(8,9). *Mycobacterium marinum* and *Mycobacterium ulcerans* are the two species that remain too close to Mycobacterium tuberculosis in terms of their fatty acid profile, DNA-DNA hybridization and 16SrRNA Gene sequence analysis(10). This helps to develop diagnostic and epidemiological tools that are species specific(10). Also its because of this reason *Mycobacterium marinum* can be used in the place of *M. tuberculosis* for new drug discovery. Surrogate model of M.tuberculosis i.e, M. marinum in Zebra fish will give us a genetically tractable natural host in which complex interactions involved in the tuberculous granuloma formation can easily be visualized. Genetic compari-

sons of the two species have given out positive results to use it as a surrogate in Zebra fish as tuberculosis disease model(11). Adult zebra fish model rather than its embryo model is better because of the information that we can generate on the data dosage, pharmacokinetics and overall health improvement(12). This surrogate model with Zebra fish infected with *Mycobacterium marinum* for Tuberculosis in humans has got now standardized protocols which could help researchers in finding new drugs especially of herbal origin with least available resources and standardized protocols(12).

### Conclusion

It has been always been a battlefield between the microbes and drug discoverers in eradicating or curing a disease. Since the beginning of the "Scientific Inquiry" man has always explored the nature through nature for the betterment of his race in this planet. Tuberculosis has succeeded through ages to persist and evolve as a disease to consume human's. It's high time that researchers need to overpower this menace before it make us all succumb to its victory.

### Conflict of Interest

None

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