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"A comparative *in vivo* and *in vitro* study on the effectiveness of Homeopathic medicines in Aquarium Zebra fish (*Danio rerio)* infected with *Vibrio parahaemolyticus".*

INTRODUCTION:

Like humans and other animals fishes are also prone to diseases from parasites. Human infections caused by pathogens transmitted from fishes and aquatic animals are quite common now-a-days. Human infections associated with *Vibrio parahaemolyticus* rank high among the pathogenic bacteria associated with fishes which cause septicemia and gastroenteritis in humans. Rather than curing the diseases in humans, we can prevent those infections in fishes by homoeopathic medicines. This will be profitable in the aquaculture and fish industries where fish is used both as food and for ornamental purpose. According to GATT (General Agreement on Table and Tariff) by World Trade Organization, it was estimated by a research team that the global aquarium industry would grow at 10-15% every year [1]. The increase in pathogenic infections will be a threat to this growing global aquarium industry. The pie chart given below shows the percentage of Vibrio infections in the world.

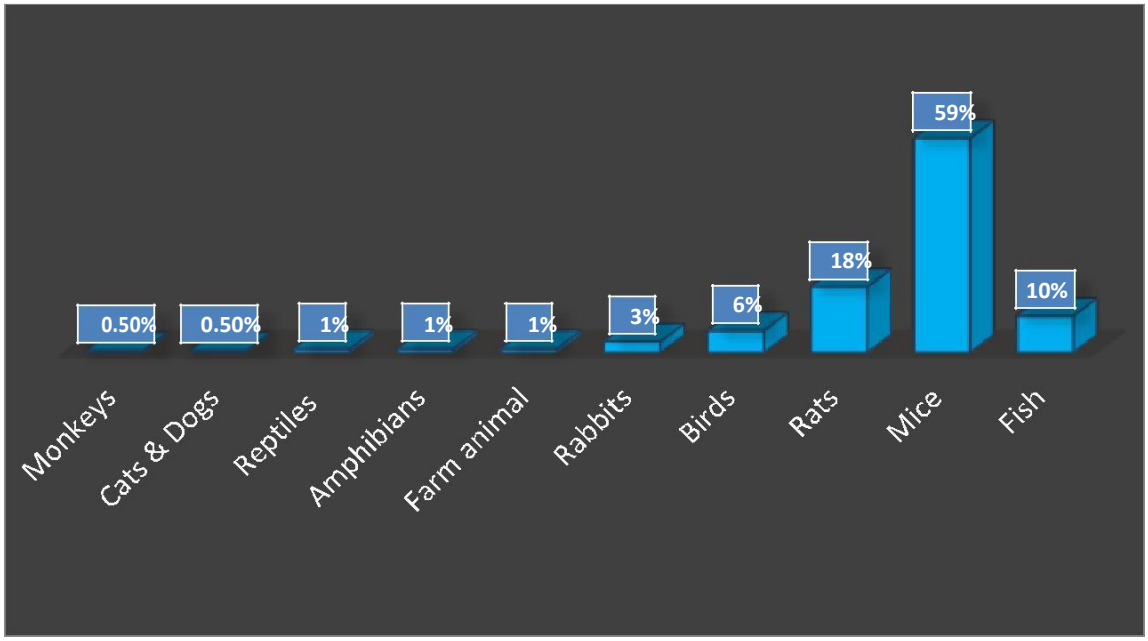
REPLACING LABORATORY ANIMALS BY FISHES:

For research purposes usually animals like rat, mice, monkeys, prosimians, cats, dogs, guinea pigs, rabbits, squirrels, etc are used. Most of the research animals are usually overfed, therefore prone to Type 2 Diabetes mellitus and renal failure. This alters the gene expression in substantial ways and leads to cognitive decline. The following bar graph shows the statistical report on the number of animals used for experimental and other scientific research purposes in the European Union, 2008. Recently, mice and other lab rats are successfully replaced by fishes for research purposes. [2].

The major advantage of using fish over mice and other lab animals are:

1. They reproduce quickly

2. They are economical
3. They are easy to maintain. [3].



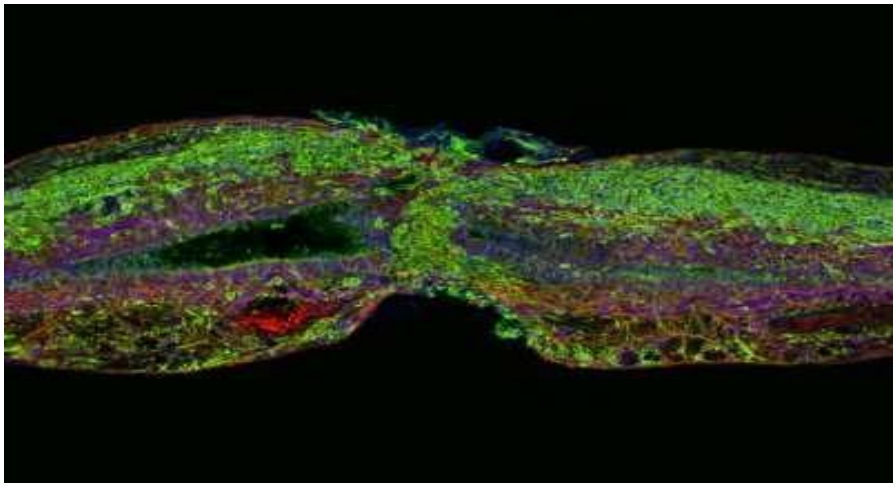
MAJOR ADVANTAGES OF USING ZEBRA FISH OVER RODENTS:

Since the scientists learned to selectively mutate zebrafish DNA in 1988, this gave them the ability to turn the species into models of human diseases. [4,5,6]. The number of biomedical zebrafish papers skyrocketed from 26 to 2100 last year. Hence, the major advantages of zebrafish over rodents are:

1) ***They reproduce quickly:*** A zebrafish female produces hundreds of embryos 3 days after fertilization while a mice takes 3 weeks to produce 10 pups.
2) ***Economical:*** Zebrafishes are economical and easy to maintain.
3) ***Larval fishes are transparent:*** Due to the transparency of the larval fishes, the researchers can literally watch the organs grow. This helps in studying the problems with organ development.
4) ***High regenerative capacity:*** Zebrafish can regenerate various parts including skin, heart, retina, brain, etc.
5) ***Toxicological studies*:** Zebra fishes prove an ideal platform for targeted studies and mechanisms of toxic action, especially Study on Lethal doses
6) ***Cytological studies & whole animal investigations:*** Due to the transparent nature of the embryos and the juvenile fishes, it is effective in whole animal studies and mechanisms of toxic action of drugs.

As far as homoeopathic medicines are considered, they are safer and effective in all living beings, they are effective in fishes also.

EVIDENCES FOR USING ZEBRAFISH IN RESEARCH:SPINAL CORD REGENERATION:



In the above given diagram, Glial cells are indicated in red, ependymal cells in green and neuronal cells are indicated in neuronal blue.

When zebra fish sustains an injury, glial cells in them create a bridge between the spinal cord tissues. These glial cells extent projections into a distance 10 times their own length that connects the gap across their injury. The injured part is completely regenerated within 8 weeks. To understand this scientists studied the genome of an injured zebra fish. It was found that the level of a protein called Connective Tissue Growth Factor (CTGF) was increased. This stimulated the regeneration of spinal cord. Humans also have this protein that can boost regeneration but the process of regeneration is complex in humans. [4].

AIMS AND OBJECTIVES

The primary aim of the study is to show the effectiveness of Homoeopathic Medicines in fish diseases.

Objectives of the study are:

- ✓ To cure the symptoms presented by *Danio rerio (Aquarium Zebra fish)* affected with *Vibrio parahaemolyticus*
- ✓ To show the effectiveness of homoeopathic medicines '*in vitro'* on fish pathogen *Vibrio parahaemolyticus*
- ✓ To compare the growth inhibition of the growth sensistivity in '*in vitro'* and '*in vivo'* studies.
- ✓ To demonstrate the effectiveness of Homoeopathic Medicines over Allopathic Medicines.

REVIEW OF LITERATURE:

According to Dr Hahnermann, disease is a varying condition of life principle which not only creates but controls an organism. Homoeopathic remedies cover all the phenomenon of the disease of whatever origin it may be ever to the microorganism. After the administration of a homoeopathic remedy, when the principle life force is resumed, it puts an end to the existence of the morbid microorganism .[8] After many years of investigation, the cause of the disease was found to be an ancient, universally diffused, contagious infectious principle present in the parasitical microorganism, called '*Psora*' meaning '*itch'*. It has the capacity for multiplication and growth. The manifestations of psora are itching and burning in the skin and the external parts. [7]

When a pathogenic organism enters a living organism, a resistance is offered, which is called 'disease'. Thus, disease is a vital reaction of a living organism to the influence of an agent which is inimical to its welfare. Pathologists agree that all the pathogenic microorganisms produce its effects in the living body by means of specific poisons which they secrete while living, or generate after death. Dr Hahnemann's generalization is based upon the existence of living specific infectious microorganisms as the cause of the greater part of all the disease. All drugs act by the virtue of their specific drug properties. All the bacterial diseases are primarily intoxications or bacterial diseases.

"Infection is indeed more often taken than is supported. It is generally received with the air in breathing.", said Dr Stuart Close. This clearly reveals that confused state of medical opinion during Hahnemann's time which resulted in the most startling, revolutionary and far reaching theory of ' the parasitical nature of the infectious and chronic diseases'. Dr Hahnemann by using the word ' miasm' had something more in mind than just 'an aerial fluid mixed with atmospheric air' [6].

1. A pilot study conducted by L Ganesh, P Seppan, B Anandan, of Dr. ALM Postgraduate Institute of Basic Medical Sciences, University of Madars, Chennai, India on topic entitled **Asserting the anxiolytic effect of homeopathic medicines: Gelsemium sempervirens 30C and Argentum nitricum 30C using Zebrafish**, where the investigator tested tropical fresh water "Zebrafish" – *Danio rerio* with two homoeopathic drugs: Gelsemium sempervirens and Argentum nitricum in 30C, ultra-high diluted homeopathic remedies against anxiety. This shows the need for fundamental research on homeopathic remedies to trace the pharmaco-dynamics at molecular and genetic stratums [9].

2.*'Vibrio parahaemolyticus*: A CONCERN FOR FOOD SAFETY' by Yie-Sheng Su and Cheng Chu Liu in *'Food Microbiology (Volume 6)',*it is explained that *Vibrio parahaemolyticus* is a human pathogen widely distributed in marine environments isolated from a variety of raw sea food. The consumption of such contaminated sea foods may lead to the development of acute gastroenteritis characterised by diarrhoea, vomiting, nausea and abdominal cramps. This is a common food borne infection in US and many Asian countries including China, Japan and Taiwan. This study provides information about the new methods for detecting *Vibrio parahaemolyticus* infections associated with sea food consumption [10].

3.'Genomic sequence of *Vibrio parahaemolyticus*:A pathogenic mechanism distinct from that of *Vibrio cholera*' by Kozo Makino, Kenshiro Oshima, Ken KUROKWA, Katsushi Yokoyama, TakaykiUda, Kenichi Tagomori, Yoshio Iijima, MaratomoNajima, Masayuki Nakano, Atsushi Yamashita, Yoshino Kubota, Shingenobu Kimura, TeruoYasunaga, Takeshi Honda, Hideo Shinagawa, Masahira Hattori, Dr Tetsuya Lida in *The Lancet,* it was revealed that *Vibrio parahaemolyticus* strains of a few specific serotypes probably derived from a common ancestor have safely caused a pandemic outbreak of gastroenteritis. The rearrangements in the genome of *Vibrio parahaemolyticus* where compared with that of *Vibrio cholera* between the two chromosomes 3288.558bp and 1877212bp is responsible for causing inflammatory diarrhoea and septicaemia [11].

4. 'Reproductive response of the guppy fish *Poecilla reticulate* for homoeopathic medicine, Natrummuriaticum' by Sudha.C and Gokula.V in '*Bilife: An International Quarterely Journal of Biology and Life Science'* it was published that Natrummuriaticum 30 was used to induce spawning in *Poecilia reticulate* (guppy fish).This study shows the effectiveness of 0.01 % of Natrum muriaticum30 more than other concentrations (0.02%, 0.03%, 0.04%) [12].

5. 'Effectiveness of Natrum muriaticum in Induced spawning in the ornamental fish *Poeciliasphenops.'* by K.Premdas, M.Lekshmanaswamy and M.Jesikha in *'Journal of Pharmaceutical and Biological Research',* showed the effectiveness of the homoeopathic medicine Natrum muriaticum for inducing breeding in White molly or *Poecilias phenops*. Natrum muriaticum 30 of 0.025% dilution was found effective. Considerable change in

weight gain, fecundity and ammonia excretion in experimental fishes were noted in the study [13].

6.'Influence of homoeopathic product on performance and on quality flour and cookie (Grissini) of Nile tilapia' by Mariana Manfroi, Denise Pastore De Lima, Ana Paula Andretto, Leidiane Accordi Menezes, Aloisio Henrique Pereira Souza, Maria Luzia De Souza Franco, Nadia Cristina Steinmacher, Saraspathy NaidooTerroso Gama De Mendonca and Lauro Vargas, it is explained that animals can also benefit from homoeopathic remedies by having their immunological system and organic responses to stress reduction stimulated, as well as their balance re-established. The objective of the study was to assess the performance of Nile tilapia treated with food containing a homoeopathic product as well as physical, chemical, technological and sensory quality of flour and cookie based on the fish co-product. The fish treated with food containing the homoeopathic product Homoeopatila 100 presented that the total weight significantly higher than the fish in the control group [14].

7. 'Application of Homoeopathic Drugs in Ornamental fish maintanace-Effect of Natrum muriaticm on Gold Fish: A Case Study' by Dr Sreekumar.S, explains about 3types of studies conducted in gold fish.

1) The growth studies indicate that the fishes treated with Natrum mur showed an excellent growth perfo4rmance over the control tanks i.e. upto 166.6% in 40 days observation.
2) The survival rate of the fishes treated with Natrum muriaticum was high upto 91% whereas it was 67% for the control.

From the reproductive behavior, it was found that the size of eggs of fishes treated with Natrum muriaticum was larger than that of the control and spawning and number of offsprings were also increased in fishes treated with Natrum muriaticum [15].

8. 'VIBRIOSIS IN FISH AND ITS CONTROL' by C.B Munn in 'Aquaculture Research' explains the symptoms of vibriosis are ulcers, lethargy, loss of appetite with red spots on the ventral and lateral areas of the fish, swollen and dark skin lesions that ulcerate, releasing bloody pus (starting as reddening or blood streaks under the skin surface).Eye symptoms include cloudy eye leading to popeye and eye loss. Course of the infection is rapid. Most of the infected fish die without showing any further clinical signs than the ulcers. The

medicines that are given for vibriosis are Oytetracycline, nifurpirinol, Myxazin, Potassium permanganate [16].

9. 'Vibriosis' by G.L Bullock in '*Conservation in Action by Bear River*' , the clinical signs of Vibriosis i.e. hemorrhage to intestines, body cavity, spleen and muscles, distended mucoid and necrotic intestine, petechiation, erosion, darkened colouration to the skin and fins are explained. It also includes changes to the eyes which are distention, cloudiness and periorbital swelling. White or grey lesions can be found on the intestines and spleen and in fry, spleenomegaly can be seen. Disease outbreaks can be influenced by water quality and temperature, the strain and virulence of Vibrio and the amount of stress imposed upon the fish [17].

10.'*Vibrio parahaemolyticus:* CELL BIOLOGY AND PATHOGENICITY DETERMINANTS' by Christopher A Broberg, Thomas J Calder and Kim Orth in '*Microbes and Infections',* it is explained that *Vibrio parahaemolyticus* is a significant cause of gastroenteritis worldwide characterization of this pathogen has revealed a unique repertories of virulence factors that allow for colonization of the human host and disease. This article describes the known pathogenicity determinants while establishing the needs for continued research [18].

11.'ZEBRA FISH:FROM DRUG DISCOVERY TO DISEASE MODELLING' by Amy .L. Rubinstein' in '*Current opinion in the Drug discovery and development'* published in 2003, throws light to the fact that zebra fish models can be used for studying diabetes, muscular dystrophy, neurodegenerative diseases, diabetes, blood diseases, angiogenesis and lipid metabolism. Those genes that are defective in each of the conditions in humans can be found in zebra fish which shows similar disease manifestations [19].

12.'HEART REPAIR REGENERATION:RECENT INSIGHTS FROM ZEBRA FISH STUDIES' by Chin-Ling Lein, Michael R. Harrison, Tai-Lan Tuan, Voughn A Starnes published in '*Wound repair and regeneration-The international journal of tissue repair and regeneration'* suggests that cardiovascular disease is the leading cause of death worldwide. In contrast to humans and other mammals, the heart of zebra fish regenerates after substantial injury or tissue damage. In this study, the recent progress in studying zebra fish heart regeneration is explained. Also a comparison of different injury models utilized to study zebra fish heart regeneration and the differences in responses to injury between mammalian

and zebra fish hearts was discussed. By learning how zebra fish hearts regenerate naturally, we can better design therapeutic strategies for repairing human hearts after myocardial infarction. This will help to replace congenital conditions in humans [20].

13. 'Experimental infection model for shrimp vibriosis studies: A review' by Denis Saulmer, Phillipe Haffner, Cyrille Goarant, Peva Lary, Dominique Ansquer in *'Aquaculture'*, it was explained that Vibrio species have become a major source of concern for shrimp culture because of their close association with low survival rates in hatcheries or grow out ponds. This review presents the usefulness of infection models with vibriosis pathogens for their pathogenicity experiments, testing of curative or prophylactic treatments and the study of the host factors influencing bacterial virulence [21].

14.'Comparative pathogenesis of bacteria causing infectious diseases in fishes.' by Ponnerassery S.Sudheesh, Aliya Al-Ghabshi, Nashwa Al-Mazrooei and Saoud Al-Habsi, it was explained that Vibrio are mainly pathogenic to freshwater and brackish water fish. The distribution of vibrio infection is worldwide and cause great economic loss to aquaculture industry. It causes septicaemia and winter ulcer in fishes. The study also reveals about the genomic differences in variety of vibrio[22].

15. 'Fish diseases transmitted to humans' by Adrian Lawler, he explains that *Vibrio parahemolyticus* causes gasterointestinal infections that can lead to rapid dehydration and systemic infections if bacteria enters blood. Antibiotics that are utilised have been tetracycline, penicillin, ampicillin, gentamycin, etc [23].

16. 'Pathogenesis responsible for seafood associated infections' in *Clinical microbiology review* suggests that *Vibrio parahaemolyticus* and *Vibrio vulnificus* are the species that are commonly associated with reported infection. Clinical features of *Vibrio parahaemolyticus* infection are watery diarrhoea, abdominal cramps, nausea, vomiting, wound infections and septicaemia. The laboratory diagnosis is made by the isolation of the organism from blood, stool and wound samples. They are overlooked upon standard agar plates in TCBS (Thiosulfate Citrate Bile salt Sucrose) media. It also includes the preventive

measures for the prevention of Vibrio infection [24].

17. 'Gobal dissemination of Vibrio parahaemolyticus SerotypeO3:K6 and its serovariants' by G.Balakrish Nair, Thandavarayan Ramamurthy, Sujith K.

Bhattacharya, Basabjit Dutta, Yoshifumi Takeda and David A.Sackdepicts the

incidents of Vibrio parahaemolyticus infection in various parts across the globe. Food borne outbreaks were found in Calcutta and sporadic outbreaks were found in Bangladesh, Chile, France, Japan, Korea, Peru, Laos, Mozambique, Russia, Spain, Taiwan, Thailand and US[25].

18. **'Fish: A potential source of bacterial pathogens for human beings' by L. Novotny, L. Dvorska, A. Lorencova, V. Beran and I. Pavlik** in '*Czech Academy of Agricultural Sciences'* explaines *Vibrio parahaemolyticus* has been isolated from sea and estuary waters on all continents with elevated sea water temperatures. *Vibrio parahaemolyticus* cause acute gastroenteritis that is self limiting. Fish food associated with illnesses due to the consumption of *Vibrio parahaemolyticus* includes fish balls, fried mackerel, tuna and sardines. These products include both raw and undercooked products that have been substantially recontaminated. Also incidents of Vibrio outbreaks in Japan in 1950s, and those of Taiwan and Japan in 1999, USA in 1970s were reported[26].

INDICATIONS OF HOMOEOPATHIC REMEDIES USED:

The most common symptoms presented by vibriosis are gasteroenteritis and septicemia. With reference to various homoeopathic literatures, the prominent medicines for gasteroenteritis and septicemia are Sulphur, Arsenicum album and Pyrogen.

SULPHUR:

'BOERICKE'S NEW MANUAL OF HOMOEOPATHIC MATERIA MEDICA WITH REPERTORY' by William Boericke, [29]. the following were the indications given for Sulphur:

a) This is the great Hahnemann's anti psoric
b) Elective remedy for skin
c) Dirty, filthy people prone to skin affections
d) When carefully selected remedies fail to act, especially in acute diseases, it frequently arouses the reactive powers of the organism.
e) Burning ulcer at the margin of eyelids(ulcerative blepharitis)
f) First stage of chronic blepharitic ulceration
g) Stool: frequent, unsuccessful desire for stool, hard, knotty, insufficient.
h) Skin: Dry, scaly, unhealthy, every little injury suppurates
i) Excoriation especially in the folds

ARSENICUM ALBUM:

'BOERICKE'S NEW MANUAL OF HOMOEOPATHIC MATERIA MEDICA WITH REPERTORY' by William Boericke, [27].

a) Acts on every organ and tissue.
b) The most prevailing symptoms are exhaustion, debility and restlessness with nocturnal aggravation.
c) Great exhaustion after the slightest exertion
d) Irritable weakness
e) Sea side complaints
f) Ailments from decayed food or animal matter.
g) Reduces the refractive index of blood serum.
h) Septic fevers and low vitality.
i) Great anguish and restlessness
j) Changes places continuously
k) Skin: dirty, rough, sensitive, covered with dry scales, very sensitive.
l) Eyes: Eyelids are red, ulcerated, scabby, scaly, granulated; Edema around the eyes; External inflammation; Corneal inflammation; Intense photophobia;
m) Stomach extremely irritable; faintness, icy coldness, extremely irritable.
n) Stool: small, offensive, dark.
o) Skin: Itching, burning, eruptions, popular, dry, rough, scaly, edema, swellings; epithelioma of skin; ulcers with offensive discharge; Gangrenous inflammations.

PYROGEN:

'BOERICKE'S NEW MANUAL OF HOMOEOPATHIC MATERIA MEDICA WITH REPERTORY by William Boericke'[28].,

The following are the indications:

a) Pyrogen is a great remedy for septic states with intense restlessness.
b) "In septic fevers, especially puerperal, Pyrogenium has demonstrated its great value as a homoeopatic dynamic antiseptic", said Dr.H.C Allen.
c) Mind: full of intense anxiety and insane notions.
d) Stool: brown black painless offensive.
e) Skin: A small cut or injury becomes very swollen and inflamed, discolored, dry.

METHODOLOGY

ACCLIMATISATION PERIOD:

15 pairs of healthy Aquarium Zebra fishes were purchased from Sneha Aquarium at Kulasekharam in Kanyakumari district of Tamil Nadu state

The fishes were given an acclimatization period of one month from 21/1/2017 to 21/2/2017.

Age of the Zebra fishes when brought on 21/1/2017 was 3 weeks. All the 30 fishes were put in the same tank.

The general features of the fishes were noted down for a period of one month.

The fishes were fed with frozen shrimp once in every alternate day. Fish food was brought from Arjun Pets and Aquarium, Near Cosmopolitan Hospital, Pattom P.O, Trivandrum-4.

GENERAL FEATURES OF FISHES ON 21/1/2017:

a) The fishes are all transparent

b) Fishes are alert and respond to the movements outside the tank.

c) Fins: Healthy fins that flow as they swim.

d) Gills: Healthy gills that can open and close with ease.

e) Eyes: Healthy clear eyes.

f) Cardiovascular system: The area of heart and blood vessels are marked by their reddish colour and can be easily observed due to the transparency of the skin.

g) Length of the fish=2 to 2.5 cm

h) Compressed body i.e. laterally flattened.

i) Photophobia^{+++}.

PHYSICAL PARAMETERS:

P^H of water:7.51(approximate)

Temperature: 25-30ºC (Room temperature)

AQUARIUM SETUP:

The healthy Aquarium Zebra fishes were divided as 6 fishes per tank. The tanks are made up of glass with dimensions: 1 ft x1ft x 1ft

- ✓ The bottom of the tanks were filled with clean stone chips and a platform filter set up.
- ✓ Proper aeration was provided.
- ✓ The tank was filled with 2/3rd water from the top.
- ✓ The fishes were placed along with their plastic covers for some time in the tanksinorder to equalize the temperature.

➢ **DIFFERENCE BETWEEN MALE AND FEMALE ZEBRAFISH:**

MALE ZEBRA FISH	**FEMALE ZEBRA FISH**
	
Slender and rather sleek	Rounded
Swelling in the abdominal region is absent	Swelling in the abdominal region is present
Smaller than female zebra fish	Larger than male zebra fish
Pinkish and yellowish colored when mature i.e. presence of golden tones	Blue and white covering when mature i.e. presence of silver coverings
Chases females in early morning	No chasing of males

***IN VIVO* STUDY:**

1. **INOCULATION OF '*Vibrio parahaemolyticus*':**

Inoculation of Vibrio parahaemolyticus

Mixing of Vibrio parahaemolyticus with feed- Impregnation technique

- ✓ The bacteria, *Vibrio parahaemolyticus* was inoculated into fish food frozen shrimp) by **'Impregnation technique'** at 1:00 pm on 21/2/2017.
- ✓ Sterilize the inoculation loop and the mouth of the test tube with the *Vibrio parahaemolyticus* culture in nutrient broth using a spirit lamp in a laminar airflow.
- ✓ 1 ml broth culture of *Vibrio parahaemolyticus* was taken in a micropipette and inoculated into 500 grams of fish food (frozen shrimp) and mixed thoroughly and incubated for 20 min at 28ºC before being used for infecting experimenting tanks.
- ✓ From the above mixture, two spatulas (~ 100 gm) of the contaminated food were mixed with the water in each tank (at 1:30 pm).

2. **NOTING DOWN THE SYMPTOMS IN CHRONOLOGICAL ORDER:**

- ✓ The symptoms were noted down from the time of inoculation.
- ✓ In all the tanks, the fishes showed marked restlessness at the time of inoculation. This was their natural response to any external stimuli.
- ✓ Fishes were checked after 1and 3 hours. But no unusual symptoms were observed.

3. **APPEARANCE OF SYMPTOMS:**

- ✓ *After 6 hours,* symptoms started appearing.
- ✓ The parameters that were noted include the following:

1) Movement and restlessness
2) Changes in skin
3) Changes in gills
4) Changes in fins
5) Characteristics of stool
6) Dwelling zone
7) Nature of water

- ✓ Most of the fishes in Tank A showed the symptoms of Sulphur, Tank B showed Pyrogen and Tank C showed Arenicum album.
- ✓ The symptoms were noted at 7:00 pm on 22/2/2017.

4. **ADMINISTRATION OF HOMOEOPATHIC MEDICINES:**

- ✓ Symptoms were noted at 7:30 pm on 23/2/2017.
- ✓ Based on symptomatology, homeopathic medicines were administered at 7:50 pm on 23/2/2017.
- ✓ In Tank A, Sulphur 200/20 drops were administered.:

a) Slowness of movements
b) Stool: Thick dark colored stool in broken pieces.

- ✓ In Tank B, Pyrogen 200/20 drops were administered.

a) Fishes donot move in shoals.
b) They visit the top zone and the middle zone repeatedly.
c) The lateral fins are stuck together and it requires great strain to free them.

d) Movement requires great strain and is slow paced.

- ✓ In Tank C, Arsenicum album 200/20 drops were administered.
  a) Marked restlessness is observed.
  b) Some move in shoals. Some are detached from their shoals and are moving alone.
  c) The fishes move more in the bottom zone and rarely visit the other zones. This indicates that they are stressed.
  d) Stool: White colored slimy stool.
- ✓ In Tank D, Tetracycline/5drops was administered as a NEGATIVE control. Tetracycline is an indicated allopathic antibiotic for bacterial infections.
  a) Slow paced movements.
  b) The fishes dwell more in the bottom zone and visit the middle zone frequently.
- ✓ In Tank E, no medicine was administered. This is used as a POSITIVE control.
- ✓ The medicines Sulphur, Pyrogen, Arsenicum album and Tetracycline was administered in the tanks A, B, C and D respectively.

5. **AFTER THE ADMINISTRATION OF FIRST DOSE OF MEDICINES:**

- ✓ As an immediate response, marked restlessness was observed in all the tanks.
- ✓ The observations were noted at:
  A) At 8:00 pm on 24/2/2017.
  B) At 8:00 pm on 25/2/2017.
  C) At 8:00 pm on 26/2/2017.

6. **FIRST REPETITION:**

- ✓ At 7:30pm on 27/2/2017, all the medicines were repeated i.e. Sulphur 200/20 drops, Pyrogen 200/20 drops, Arsenicum album 200/20 drops and Tetracycline/5 drops were administered in Tanks A, B, C and D respectively.
- ✓ The observations were noted from the time of administration of medicine.
- ✓ No immediate response was observed this time in any of the 4 tanks.
- ✓ Also, observations were noted at 7:30pm on 28/2/2017.

7. **SECOND REPETITION:**

- ✓ At 7:30 pm on 1/3/2017, the symptoms were noted and all the medicines were repeated again i.e. Sulphur 200/20 drops, Pyrogen 200/20 drops, Arsenicum album 200/20 drops and Tetracycline/5 drops were administered in Tanks A, B,C and D.
- ✓ The observations were noted at the following time intervals:

1. At 7:30 pm on 2/3/2017
2. At 7:30 pm on 3/3/2017
3. At 7:30 pm on 4/3/2017
4. At 7:30 pm on 5/3/2017

8. **COMPARISON OF THE OBSERVATION IN '*IN VIVO*' METHOD:**

1. The rate and duration of cure individually and collectively from all the 3 tanks in which homeopathic medicines were administered
2. The rate and duration of cure in the case of treatment with homoeopathic medicines over allopathic medicines
3. The rate and duration of cure in case of homeopathic medicines over natural cure.

***IN-VITRO* STUDY:**

1. SERIAL DILUTION OF WATER:

i. ***Requirements and preparation:***

Clean conical flasks, Double distilled water, Test tubes, cotton, Electronic weighing balance, Measuring cylinder, TCBS Agar, Nutrient agar, Agar plates, Laminar air flow, Spirit, Induction top, Induction cooker, label and permanent marker.

Label the conical flasks as Tank A, Tank B, Tank C, Tank D and Tank E respectively.

Collect 10ml of water sample each from all the 5 tanks using a bulb pipette in the respective conical flasks.

Clean the laminar air flow with spirit and cotton.

Make cotton plugs for all the 5 conical flaks and 5 test tubes.

Sterilize 250ml of double distilled water each in an induction cooker at a temperature of 1100 degree Celsius for 15 minutes in all the conical flasks.

Also take 100ml distilled water in other 2 conical flasks and sterilise it at a temperature of 1100 degree Celsius for 15 minutes in an induction cooker.

Also sterilize 10 agar plates at 1100 degree Celsius in an induction cooker.

Label the agar plates as TANK-A: SULPHUR 200, TANK B- PYROGEN 200, TANK C- ARS ALB 200, TANK D- TETRACYCLINE 200 and TANK E- CONTROL.

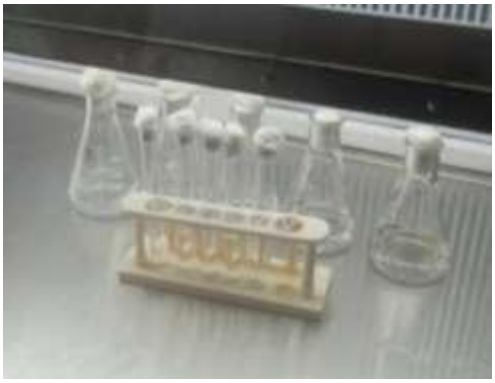
Serial dilution:

Step :1 Taking water sample for serial dilution

Step : 2 Measuring 10 ml water sample using a Pasteur pippette

Step : 3 Transferring the contents from sample tubes to dilutions

Step : 4 Completed dilutions



Step : 5 Labelling and storing tubes

serial dilution (cont…d)

10 ml of water from each tank (A –E) was taken in a clean sterilized cotton plugged test tube. Under aseptic condition 1 ml of water sample from each test tube was transferred to test tube labeled 10^{-1}containing 9 ml of sterilized water using a micropipette and the process was repeated till 10^{-6}.

ii. Preparation of media:

Weigh 8.98 grams of TCBS agar using an Electronic balance and add it to one of the conical flasks with 100ml of distilled water and mix it well using a stirrer.Thus, TCBS media is prepared.

Also weigh 5.85 grams of Nutrient agar using an electronic balance and add it to one of the conical flasks with 100 ml of distilled water and mix it well using a stirrer. Thus, Nutrient media is prepared.

The mixed media is kept for heating at 1100 degree Celsius for 15 minutes in an induction cooker.

iii. Preparation of plates:

The plates are prepared using Pour Plate Method.

1 ml each of 10^{-4},10^{-5}and10^{-6}dilution from test tubelabeled A is taken using a micropipette and transferred to the sterilized Petri dishes labeled A 10^{-4}, A 10^{-5}, A10^{-6} , on to which 15 to 20 ml of media was dispensed. The plates were labeled TANK A-SULPHUR 200.

Similarly, 1 ml of each of the 10^{-4},10^{-5}and10^{-6} dilution from the test tubes labeled B,C,D and E are transferred using a micropipette and transferred to the sterilized agar plates labeled TANK B-PYROGEN 200, TANK C-ARSENICUM ALBUM 200, TANK D-TETRACYCLINE and TANK E-CONTROL.

All the plates are moved gently so that the medium and the water sample get mixed.

The plates are kept undisturbed for the time till they get solidified.

iv. Incubation:

After solidification, the plates are kept in the incubator for 24 hours at room temperature.

2. CHECKING THE GROWTH INHIBITION IN THE MEDIAS:

The TCBS agar plate shows the rate of growth inhibition

The Muller Hinton agar plate shows the rate of growth inhibition

The growth inhibition in the media is checked for the comparison of:

1) Different homeopathic medicines.
2) Homoeopathic medicines over allopathic medicines.
3) Homoeopathic medicines over control

Requirements:

1) TCBS medium
2) Nutrient medium
3) Muller Hinton Agar plates
4) Sulphur 200

5) Pyrogen 200

6) Arsenicum album 200

7) Tetracycline

8) Discs

Label the agar plates on its four corners as Sulphur 200, Arsenicum album 200, Pyrogen 200 and Tetracycline and labelthe centre area as Control.

Add 20ml of TCBS media to an agar plate. Similarly, also add 20 ml of the nutrient media to the other plate.

Wait till the media solidifies in boththe agar plates.

Soak 2 discs each in 3 to 4 drops of Sulphur 200, Pyrogen 200, Arsenicum album 200 and Tetracycline.

Place the soaked discs in the respective labeled areas in both the agar plates. Keep the plates for incubation at room temperature for 24 hours.

3. EXAMINATION OF GUT OF FISH:

The TCBS media shows the presence of Vibrio at the rims of the plate

Gut of the fishes from the tanks was taken and checked for the presence of *Vibrio parahaemolyticus.*

It was taken after careful dissection of the fish using forceps and blade.

The gut thus taken was mixed in distilled water and then serial dilution of the gut was done.

1ml of the distilled water mixed with gut of the fish was dropped into the conical flask with 250ml distilled water using microtip and micropipette.

1ml from this mixed water was taken and transferred into the agar plate.

20ml of the TCBS Agar media was poured over it and kept for solidifying.

Similarly, 20ml of Nutrient agar media was poured in another Petri dish and kept for solidifying.

Then the media is kept for incubation for 24 hours at room temperature.

OBSERVATION CHART:

Date and time of inoculation: 1:00 pm on 22/2/2017

Date &Time	Tank A:	Tank B:	Tank C:	Tank D:	Tank E:
7:00pm on 22/2/17	1)Slowness of movement 2)No notable change in the skin 3)No notable change in gills 4)No notable change in fins 5)Stool: couldn't find 6)Fishes move in the top zone and rarely visit the bottom zone.	1) Movement requires great strain and is slow paced. Fishes do not move in shoals. 2) No notable change in the skin. 3)No notable change in gills 4) The lateral fins have stuck together.They try to free the fins that are stuck together. 5)Stool: couldn't find 6)Visit the top zone and the middle zone regularly but the bottom zone is visited rarely	1)Fishes move in shoals 2) No notable change in the skin 3)No notable change in gills 4)No notable change in fins 5)Stool: couldn't find 6)They move more in the bottom zone and the middle zone and rarely visits the top zone	1) The movement of the fishes are slow paced. 2)No notable change in the skin 3) No notable change in gills 4)No notable change in fins 5)Stool: couldn't find 6)Fishes visit all the zones equally.	1) Movement requires great strain and is slow paced. 2)No notable change in the skin 3) No notable change in gills 4)No notable change in fins 5)Stool: couldn't find 6)They move more in the bottom zone and the middle zone and rarely visits the top zone
7:00pm on 23/2/17	1) Slowness of movements^{+++} 2) Whitish ulcers seen in patches^{+++}. 3)Bleeding gills	1) Slow paced movement^{++}. Some of them stay at one place for a long time. The	1)Fishes move in shoals. Restlessness is present^{+++}.	1)Slow paced movement^{++}. 2)Reddening of gills	1)Slowness of movements^{++}. Fishes move in shoals.

	4)Stool: Thick dark colored stool in broken pieces. 6)Decay of fins 7) Fishes stay in the corner and rarely move.	heads are facing downwards and the tail facing upwards 2) No notable change in the skin3) The lateral fins stick together in most of them. 5)Stool:No notable change 6) Some stay in the top zone and the others are in the middle zone.	2)No notable changes in the skin 3)Stool: White colored slimy stool. 6) Decay of fins$^{++.}$ 7)Bleeding like spots can be seen in the eyes and gills^{++}	4)No notable change in fins 5)Stool: couldn't find 6)Visits the mid zone frequently 7)Skin ulcers can be seen^{++}	2)No notable changes in the skin. 3)No notable changes in the gills 4)Stool:Couldn't find. 5)Visits the bottom and mid zone more frequently. 6) Decay of fins.

At 7:50pm on 23/2/17, homoeopathic medicines Sulphur 200, Arsenicum album 200 and Pyrogen 200 were administered in Tanks A,B and C respectively. Also tetracycline was administered in Tank D. Tank E was kept as a positive control without the administration of medicines.

Date &Time	Tank A: Sulphur 200	Tank B: Pyrogen 200	Tank C: Arsenicum album 200	Tank D: Tetracycline	Tank E: No medicine
8:00pm on 24/2/17	1)The movements are speedy. The rhythm of movement returned. 2) Whitish ulcers seen in patches^{+} 3) Bleeding gills better.	1)The speed of the movement increased. Fishes restore their position and directtheir head upwards and tail downwards. 2) No notable change in the skin 3)No notable change in gills	1)The speed of the movement increased. 2)No notable change in the skin 3)Bleeding spots in eyes and gills^{+} 4)Decay of fins^{+} 5)Stool:Couldn't find	1)Fishes continue their slow pace. 2) No notable change in the skin 3)No notable change in gills 4)The efforts to move their fins are at the maximum.Thefishes with attached	1)1of the fishes was found to be dead. The others showed slow paced movement. 2)No notable change in the skin.

	4)No notable change in fins 5)Stool: Thick dark colored stool in broken pieces. 6) Stays in the top zone and visits the mid zone rarely	4)Efforts to open their fins also increases^{++}.The fins open upto a greater extent than before. 5)Stool:No notable change 6) Some stay in the top zone and the others are in the middle zone.	6) Some stay in the top zone and the others are in the middle zone.	tail fins cannot move their fins. 5) Stool: couldn't find 6)The fishes visit the top zone and rarely visits the middle zone	3)Reddening of gills present. 4)All the fishes have their fins stuck together and the efforts to free them continue.
8:00pm on 25/2/17	1)The movements are speedy. The rhythm of movement returned. 2) No notable change in the skin 3) Reddened gills without bleeding. 4)No notable change in fins 5)Stool: Thick dark colored stool in broken pieces. 6)Fishes visit the mid zone and the top	1)Two fishes are dead. The dead fishes were found at the bottom zone 2)The dead fishes have whitish discoloration on their skin.Thecolour of the skin fades in the live ones.The whitish raised areas in the skin resemble cotton balls. They showed clouded eye. 3)No notable change in gills 4)Efforts to open their fins continue. 5)Stool:couldn't find 6)The fishes move in the bottom zone.	1)All the fishes move ina normal pace. 2) No notable change in the skin 3) Bleeding spots better but persists. 4)No notable change in fins 5)Stool:Couldn't find. 6)Every one of them visits the top zone and the middle zone.	1)Fishes continue their slow pace. 2) No notable change in the skin 3)Reddening andbleeding gills. 4) The efforts to move their fins continue. 5) Stool:couldn't find 6)All of them remain in the bottom zone and visits the mid zone rarely.	1)One more fish was fund to be dead. The dead fishes are found at the bottom of the tank. 2)'Cotton ball' like appearance was found in the skin of the fishes.These are raised whitish areas in the skin of fishes. 3)Bleeding of gills is prominent. 4)Sticking together of fins

	zone more frequently.				continues and the efforts to free them persists. Fin rot is observed. 5)Stool:Couldn't find. 6)The fishes move in the bottom zone.
8:00pm on 26/2/17	1) Speed of the movements still persists. 2)No notable change in the skin 3)Bleeding of gills 4)No notable change in fins 5)Stool: Couldn't find 6)Fishes move more in the bottom zone	1)The speed of movement is reduced to a great extent 2)Skin show white patches 3) Prominent bleeding in gills. 4)Fin rot is observed in all of them. Tail fins and the lateral fins are torn. 5)Stool:couldn't find 6)The fishes move in the bottom zone.	1) The movement of two of the fishes is very slow. 2) No notable change in the skin 3)Fishes among the 6 showed bleeding gills. 4)No notable change in fins 5)Stool:Couldn'tfind. 6)These 2 fishes remained in the bottom zone. Others remain in the middle zone and visit the top zone frequently	1)Fishes continue their slow pace. 2) No notable change in the skin 3)Bleeding of gills 4)The efforts to move their fins continue. 5) Stool:couldn't find 6)All of them remain in the bottom zone and visits the mid zone rarely	1)Speed of the movement is reduced .No dead fish found. 2) Discoloration ofskin.Whitish appearance persists. 3)Bleeding of gills. $^{+++}$ 4)Stool:whitish stool mixed with blood. 5)All of them remain in the bottom zone.

At 7:30pm on 27/2/17, all the medicines were repeated i.e. Sulphur 200/20 drops in Tank A, Arsenicum album 200/20 drops in Tank B, Pyrogen 200/20 drops in Tank C, Blue medicine/ 5 drops in Tank D were administered.

Date &Time	Tank A: Sulphur 200	Tank B: Pyrogen 200	Tank C: Arsenicum album 200	Tank D: Tetracycline	Tank E: No medicine
7:30pm on 28/2/17	1)The speed of the movement increased. 2)Bleeding gills better. 3)No notable change in fins 4)Stool: Couldn't find 5)Fishes move in the top zone and mid zone.	1)1 fish is dead today.The dead fishes appear at the menisci in the top zone. Imbalance in movement persists 2)Fishes still have whitish patches on their skin 3)Bleeding gills persists. 4)The lateral fins are not at all moving 5)Stool:Couldn't find 6)They move to the top zone and show gasping like movements 7) The water in the tank has become cloudy and frothy	1)Normal pace in the movement of fishes. 2)No notable change in the skin 3)Bleeding gills better. 4)No notable change in fins 5)Stool:Couldn't find. 6)Fishes move in the top zone and mid zone.	1)Imbalance of movement.Fishes are still slow paced 2)The fishes are pale 3)Bleeding gills still persists 4)The efforts to move their fins continues. 5)Stool: Couldn't find 6)The fishes visited the mid zone and the top zone.	1)All the fishes are dead. 2)The dead fishes showed marked discoloration of skin. 3)Bleeding gills were observed. 4)Fin rot prominent. 5)Stool:Couldn't find. 6)The dead fishes were found at the bottom zone. 7)The water was was cloudy.

At 7:30pm on 1/3/17, all the medicines were repeated i.e , Sulphur 200/20 drops in Tank A, Arsenicum album 200/20 drops in Tank B, Pyrogen 200/20 drops in Tank C, Blue medicine/ 5 drops in Tank D were administered.

Date &Time	Tank A: Sulphur 200	Tank B: Pyrogenium 200	Tank C: Arsenicum album 200	Tank D: Tetracycline	Tank E: No medicine
7:30pm on 2/3/17	1)The movement is in a normal pace. 2) No notable change in the skin 3)Reddening persists. 4)No notable change in fins 5)Stool: Couldn't find 6)Fishes move in the top zone and mid zone.	1) The movement is very slow paced. 2)Fishes still have whitish patches on their skin 3)Bleeding gills persists. 4)The lateral fins are not at all moving 5)Stool:Couldn't find 6)The movement of the fishes are confined more to the bottom zone	1) Normal pace in the movement of fishes. 2)No notable change in the skin 3)Slight reddening of gills persists. 4)No notable change in fins 5)Stool:Couldn't find 6)The fishes remain in the mid zone and top zone.	1)Slow paced movement 2)Normal coloration of skin 3)Bleeding still persists. 4)The efforts to move their fins continues. 5)Stool: Couldn't find 6)Fishes move more in the bottom zone.Visits the mid zone and the top zone occasionally	----------
7:30pm on 3/3/17	1)Normal swift movement 2) No notable change in the skin 3)The gills retained their normal appearance 4)The fins are normal.	1) The movement is very slow paced. 2)The fishes show marked emaciation. Whitish 'cotton like mass' disappeared.The whitish streaks in the skin have a broken appearance	1) Normal pace in the movement of fishes. 2)No notable change in the skin 3)The gills retained their normal appearance 4)No notable change in fins 5)Stool:Couldn't find	1)Some move in shoals. 2)Normal coloration of skin 3)Bleeding of gills still persists 4)The efforts to move their fins continues. 5)Stool: Couldn't find	----------

	5)Stool: Couldn't find 6)The fishes are moving in the top zone	3)Stoppage of bleeding of gills.Reddening of gills appeared. 4)Fin rot better but still persists 5)Stool:Couldn't find. 6)Fishes move more in the top zone	6)The fishes are moving in the top zone in shoals.	6)Fishes move in the top zone and the mid zone	
7:30pm on 4/3/17	1)Normal swift movement 2) No notable change in the skin 3)The gills retained their normal appearance 4))The fins are normal. 5)Stool: Couldn't find 6)The fishes are moving in the top zone	1)One fish is dead 2)The fishes show marked emaciation. Broken appearance of whitish streaks improved. 3)Reddening of gills persists. 4)Fin rot absent 5)Stool:Couldn't find. 6)Fishes move more in the top zone	1)The fishes are moving in shoals. Normal swift movement. 2)No notable change in the skin 3)The gills retained their normal appearance 4)No notable change in fins 5)Stool:Couldn't find 6)The fishes are moving in the top zone	1)Some move in shoals; Normal swift movement. 2)Normalcolouration of skin. 3)bleeding still persists. 4)The efforts to move their fins continues. 5)Stool: Couldn't find 6)Fishes move in the top zone and the mid zone	----------
7:30pm on 5/3/17	1)Normal swift movement	1)Normal movement in fishes.	1)The fishes are moving in shoals. Normal swift movement.	1)Normal swift movement in shoals. 2)Normal coloration of skin.	----------

	2) No notable change in the skin 3)The gills retained their normal appearance 4))The fins are normal. 5)Stool: Couldn't find 6)The fishes are moving in the top zone	2)Emaciation persists Broken appearance completely disappeared. 3)Gills are normal. 4)Fins are normal. 5)Stool:Couldn't find. 6)Fishes move more in the top zone	2)No notable change in the skin 3)The gills retained their normal appearance 4)No notable change in fins 5)Stool:Couldn't find 6)The fishes are moving in the top zone	3)Bleeding of gills still persists. 4)The efforts to move their fins continues. 5)Stool: Couldn't find 6)Fishes move in the top zone and the mid zone	

The fishes were watched for another 20 days till 25/3/2017.No fishes were found dead in Tank A and C.4fishes were found dead and 2 of them are still alive and healthy in Tank B.

RESULT:

1. ***Curing of Vibriosis:***

The incubation period of Vibriosis was 4 days.

The condition was completely cured within 10 days of administration of homoeopathic medicines used.

But in the case of tetracycline, the symptoms disappeared after 15 days.

2. ***Serial dilution of water sample:***

TANK	RESULT	INFERENCE
A		In the figure given aside, The yellow colored colonies are *Vibrio parahaemolyticus.* Although Tank A contains *Vibrio parahaemolyticus*, the fishes showed complete cure.
B		The yellow coloured areas are colonies of *Vibrio parahemolyticus*. Tank B contain *Vibrio parahaemolyticus* after the administration of Pyrogen 200. 4 out of 6 fishes died in this tank. And only the remaining 2 fishes showed complete cure.
C		Tank C doesn't contain *Vibrio parahaemolyticus* after the administration of Sulphur 200. This marks the effectiveness of Sulphur as a deep acting remedy in Vibriosis.

D		Tank D contains *Vibrio parahaemolyticus* and was not as effective as homoeopathic medicines.
E		Tank E contains *Vibrio parahaemolyticus* and no cure was found in this case.

2.The effectiveness of homoeopathic medicines in in vitro study:

Arsenicum album was the most effective medicine among the 3 homoeopathic medicines used and Sulphur ranked the second best. While Pyrogen was least effective among the 3 homeopathic medicines.

Amomg the fishes administered with Pyrogen, only 2 survived among the 6.While in all other tanks where homeopathic medicines were administered,none of the fishes died.

Mortality rate in Tank A(Sulphur 200):0%

Mortality rate in Tank B(Pyrogen 200):66.66%

Mortality rate in Tank C(Arsenicum album 200):0%

3.The comparison of the effectiveness of homeopathic medicines over allopathic medicines:

Homoeopathic medicines showed cure within 10 days while Tetracycline showed cure within 15 days.

The time required for cure is more in the case of Tetracycline when compared to the homeopathic medicines.

4.The comparison of natural cure and cure by homoeopathic medicines:

No natural cure was found in Tank E, where no medicines were administered. Mortality rate was 100%.

All the fishes died of vibriosis.

This shows that homeopathic medicines are very much effective against vibriosis where a natural cure cannot be obtained.

5.Comparison of growth inhibition :

RESULT	**INFERENCE**
	In MULLER HINTON MEDIA, the comparison of zone of inhibition is as follows: Tetracycline(32mm)>Sulphur 200(10mm)>Arsenicum album200(12mm)>Pyrogenium 200(Nil) =Control (Nil)
	In TCBS MEDIA, the comparison of zone of inhibition is as follows: Tetracycline(22mm)>Sulphur 200(12mm)>Arsenicum album 200(10mm)>Pyrogen 200(Nil)=Control(Nil)

5. Pure culture of Vibrio parahemolyticus*after serial dilutions*:

For the serial dilution of gut, the fish gut is taken carefully using forceps and scissors.

The gut is mixed with 1ml of distilled water and not crushes since it is very small in size.

1ml of this contaminated water is mixed with 100 ml of distilled water and the serial dilution is taken.

By pour plate method, 1ml from this dilution is taken and poured using a micropipette and then the already prepared TCBS media is poured over it.

The agar plate is moved slightly so that the two solutions get mixed well. The plate is kept for incubation for 24 hours at room temperature.

DISCUSSION:

Vibrio parahaemolyticus are serious pathogens for animals reared in aquaculture [16]. Vibriosis, caused by infection by Vibrio sp, is one of the most prevalent disease in fishes and are widely responsible for mortality in cultured aquaculture systems worldwide [17]. Foodborne gastroenteritis in humans especially to fish eaters was contributed by *Vibrio parahaemolyticus.* Due to an increasing trend of antibiotic resistance in aquaculture many alternative methods are in use by aquaculture scientists to reduce Vibrio-related diseases. One such method is application of Homoeopathy in aquaculture. The present study has proven the efficacy of Homoeopathic medicines in 200th potency.

The indicated remedy Sulphur given to fishes in Tank A had symptoms like Skin conditions like Dry, lusterless, scaling and scabs, Filthy, dirty skin, redness and inflammation around the eyes. Other features are excoriated and dirty dingy looking skin [31].

The indicated remedy pyrogen given to fishes in Tank B had shown gastro-duodenal ulceration with peritonitis, infections originating from either a gut condition or a respiratory condition, restlessness, constantly shifts position and toxic or septic focus [32].

The indicated remedy Arsenicum album given to fishes in Tank C had symptoms like suppurations, Dry, rough, scaly, unhealthy skin, exhaustion and restlessness. Loosing of weight due to septic infections, restlessness and anxiety, weakness, severe diarrohea with blood and skin ulcers[30].

Vibrio parahaemolyticus isolated from fish gut and water samples of tank proves the presence of vibrio in them, despite the fishes are uninfected and live. The remedy which acted quickly was Sulphur 200, followed by Arsalb 200 and the slow acting remedy was identified as Pyrogen 200.

The tank in which no mortality was noted was that of Sulphur and Ars alb. The fishes in tank B (pyrogen) had 67% of mortality index when compared to other tanks. Therefore Sulphur and Ars alb rank high in the suppuration of gastro-enteritis in fishes and thus could make better results in reducing infection in human by fish borne diseases.

LIMITATIONS:

The effect of different potencies in Vibriosis might have been studied.

The organisms could have been reinfected to have check on the immunity of the fishes cured homeopathically.

Due to the small size, minute changes in the body parts could not be noted.

CONCLUSION:

From the study, it is proved that the deep acting remedy Sulphur 200 was more effective than the other two homeopathic remedies .i.e Ars alb 200 and Pyrogen 200.This study throws light to the application of aquatic homoeopathy in microbiology. The treatment of fishes with bacterial infections is usually with antibiotics. This has led to the production of antibiotic resistant bacteria. So need arises for the use of another alternative method for treatment. Homoeopathy can completely satisfy this need.

Homoeopathic medicines do not produce any resistant bacteria but improves the immunity of the living organism. This is evident from Tank C with Arsenicum album. Although the tank contains *Vibrio parahaemolyticus*, the fishes got cured. This reveals that homeopathic medicines can act as both a curative remedy and also a prophylactic one.

Whereas deep acting remedy ensures 100% cure .i.e. both the water and the fishes are cured of Vibrio. This can act as a very useful remedy for aquaculturists because it completely removes and annihilates Vibrio from both the living organism and its environment. This study will serve as a future reference to the studies related to the application of microbiology in aquatic homoeopathy.

SUMMARY:

The study, **"A comparative *in vivo* and *in vitro* study on the effectiveness of Homeopathic medicines in *Danio rerio* infected with *Vibrio parahaemolyticus"*** is the application of microbiology in aquatic homoeopathy.

After inoculation of *Vibrio parahaemolyticus*,

- ✓ Fishes in Tank A were administered with Sulphur 200:100% cure was observed; No bacteria in the tank as well as in the fish.
- ✓ Fishes in Tank B were administered with Pyrogen 200: Least cure percentage was observed;4 out of 6 fishes were dead.
- ✓ Fishes in Tank C were administerd with Arsenicum album 200:2nd highest cure was observed. Although the water in the tank contained *Vibrio parahaemolyticus,* the fishes were cured of the infection.
- ✓ Fishes in Tank D were administered with Tetracycline: The duration of cure was very slow; The water showed the presence of Vibrio.
- ✓ Fishes in Tank E were not administered with any medicine. All of them died.

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