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Antibacterial activity of *Lantana camara* leaf extract against *Aeromonas hydrophila* on culture of Mrigal, *Cirrhinus cirrhosus* (Hamilton, 1822)

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Abstract: Mrigal (*Cirrhinus cirrhosus*), one of the major Indian carp been frequently infected by a serious bacterium known *Aeromonas hydrophila*. Due to its high pathogenicity, it is taken in to consideration for the effective control. The control includes various elements such as antibiotics, probiotics and medicinal plant extracts. It is economically important to control and prevent the infection for further culture techniques. *Aeromonas hydrophila* is the virulent motile *aeromonad*, which may affect various physiological functioning of Mrigal. Usually, *A. hydrophila* occurs in poor water quality, stressors, temperature fluctuations etc. The invasion of bacteria is indicated by number of morphological and genetic changes in the fish. It is important to identify the risk factors responsible for *Aeromonas* outbreak in Mrigal fish. The antibacterial activity of *Lantana camara* is almost effective for the control of the infection in Mrigal. The ethanolic extract consists of various phytochemical components that help in the treatment of infection. We would recommend the plant extract to treat the infection in Mrigal fingerlings.

***Index Terms*: *Aeromonas hydrophila*, *Cirrhinus cirrhosus*, Ethanolic extract, *Lantana camara*, and Pathogenicity.**

Introduction

Aquaculture is the breeding, rearing, and harvesting of fish, shellfish, algae, and other organisms in all types of water environments.Aquaculture is a method used to produce food and other commercial products, restore habitat and replinish wild stocks, and rebuild opulations of threatened and endangered species (NFDB, 2019). Organisms as varied as trout, carp and tuna (i.e., finfish), shrimps, oysters (i.e., shellfish) and seaweed are grown in ponds, tanks or nets in salt, brackish and fresh water. The role of aquaculture in ensuring a consistent supply for human consumption of fish and aquatic species in general are much healthier source of protein compared to livestock.

Global fish production is estimated to have reached about 179 million tons in 2018. Overall total of 156 million tons were used for human consumption, equivalent to an estimated annual supply of 20.5 Kg per capita. The remaining 22 million tons were destined for non-food uses, mainly to produce fish meal and fish oil. Aquaculture accounts for 46 percent of the total production and 52 percent of fish for human consumption. Asia is the largest aquaculture producer of 34 percent in the year 2018. Global fish consumption increased at an average annual rate of 3.1 percent from 1961 to 2017 and increase of 1.5 percent in the year 2018. Global capture fisheries production in 2018 reached a record of 96.4 million tons, an increase of 5.4 percent from the average of the previous three years. The increase was mostly driven by marine capture fisheries, where production increased from 81.2 million tons to 84.4 million tons in 2018. The top 20 producing countries accounted for about 74 percent of the total capture fisheries production (FAO, 2020).

Aquaculture is the rearing of aquatic organisms under controlled or semi controlled conditions. It involves plant and animal rearing with the objectives of providing human food, recreational fishing, enhancement of commercially valuable stocks, recovery of endangered species and the production of bait and ornamental species. Aquaculture is conducted in ponds, raceways, cages and net-pens (Robert Stickney, 2000). Food produced in aquaculture are often more expensive than other sources of animal protein. Fish meat is generally tender because it contains less connective tissue than other meats such as beef, mutton, pork, poultry etc, and hence it is easy to chew, particularly for children and the elderly person (Tibbets, 2001). A healthy diet, high protein is necessary to ensure that growing population does not succumb to sickness and disease. Fish and other aquatic organisms fit the model for healthy source of protein (Jayasankar, 2018; NFDB, 2020).

Mrigal (*Cirrhinus cirrhosus*), a carp endemic to Indo-Gangetic riverine systems, is one of the three Indian major carp species cultivated widely in South-East Asian countries. This species has long been important in polyculture with other native

species, mainly in India. Hatchlings of Mrigal normally remain in the surface or sub-surface waters, while fry and fingerlings tend to move to deeper water. Adults are bottom dwellers. Mrigal is a highly fecund fish. Mrigal is a tropical fresh water fish. The body is elongated with depressed and obtusely rounded snout. Scales are large and cycloid. Lateral line is continuous to the centre of caudal fin base. Abdomen is rounded; body depth and head length is nearly equal. Mouth is at terminal end and wide. Body colour is usually dark grey on the back and silvery on the sides and belly. Fins are grayish; tips of pelvic, anal and lower lobe of caudal are tinged orange. Sexual maturity is attained with 1 or 2 years depending on location. Fecundity varies from 1-2 × 104 depending upon the size of the fish. In natural waters, breeding occurs during the South-West Monsoon season. The fish breed in water of 0.5 to 1.0 m (FAO, 2014).

Lantana camara is a notorious weed and a popular ornamental garden plant and has found various uses in folk medicine in many parts of the world. *Lantana camara* is mostly native to sub-tropical and tropical America, but a few taxa are indigenous to tropical Asia and Africa. *Lantana camara* is commonly known as wild or red sage. The plant is an aggressive, obligate out breeder weed, and invades vast expanses of forest area. The plant has been used in many parts of the world to treat a wide variety of disorders. *Lantana camara* produces a number of metabolites in good yields and some have been shown to possess useful biological activities (Ghisaberti *et al*., 2000; Sanjeeb kalita *et al*., 2012; Walton, 2006).

In the present study Lantana camara leaf extracts has been applied to control the infection of *Aeromonas hydrophila* on Mrigal fish at appropriate time intervals. In the culture of Mrigal fish the hydrological parameters of the control and experimental water samples were analyzed and evaluated the survival rate of control and experimental groups have been studied.

Materials and Methods

Isolation of bacteria

One or two drops of homogenized infected fish tissue samples were kept on suitable culture media plates and streaked on the surface of the agar plates with a sterilized bent 'L' glass rod. In certain cases, the samples were diluted with sterile saline to avoid over growth of the bacterial isolates. After inoculation, the agar plates were incubated at 28ºC for 24 to 48 hours along with control plates without inoculums. After the incubation period, the inoculated Petri plates were examined carefully for bacterial growth. Morphologically similar and dominant bacterial colonies were selected and streaked on nutrient agar plates to obtain pure culture. After obtaining the pure culture, isolates were preserved for further study. In the present study, preservation in semisolid medium was followed to preserve the bacterial isolates. The isolates were maintained by stab inoculation of the organism on semi solid nutrient agar in screw cap bottles. After incubation for 24 hours, the bacterial isolates were stored at 4^0C.

Isolation and identification of microbial pathogens

Aeromonas hydrophila pure strain was obtained from Aquatic Animal Health Laboratory, C. Abdul Hakeem College. Bacterial strain was further confirmed by Catalase, Oxidase, Motility test, Hemolysis and PCR using specific primer sets. 100 µl of samples were spread on Nutrient agar and TCBS agar plates. The dominant colonies were then selected and streaked on to SCDA plates to obtain pure culture for identification test. Bacterial morphology and growth of gram stained smear from pure culture of bacterial isolation, were examined by light microscopy.

Gram-staining

After 24 hours of incubation at 28ºC on Nutrient agar the organisms were stained by Hucker's modification of the Gram-stain to study the micro morphology and Gram-staining reactions of the bacterial isolates. A small amount of surface growth of the isolate was removed from SCDA medium and mixed well with a drop of sterilized distilled water on a grease free clean microscope slide with the aid of an inoculation needle to make a smear. Smear was air-dried, heat-fixed and stained with crystal violet and safranin. The stained slides were examined under the microscope (1000 X) to study the micro morphology and Gram-staining reactions of the isolates.

Biochemical Test

A small amount of growth from each culture was placed onto each clean microscope slides. Then added few drops of H_2O_2 onto the smear and mixed well with a toothpick gently. A rapid evolution of O_2 evidence by bubbling indicates the presence of Catalase enzyme. In the Oxidase test, an artificial final electron acceptor (N, N, N', N'-tetramethyl phenylene diamine dihydrochloride) is used in the place of oxygen. With a sterile swab, a small amount of organism from each plate obtained individually. One drop of reagent was poured onto the culture on each swab. Positive reactions turn the bacteria violet or purple immediately or within 10 to 30 seconds. This indicates the presence of Oxidase enzyme. Bacterial isolate was inoculated in Blood Agar plate and incubated at 28ºC for 2 hours and examined. The presence of clear zone around the colony indicates the hemolysis of blood cells by the pathogens.

16S rRNA GENE OF *AEROMONAS HYDROPHILA* ANALYSIS BY PCR

EXTRACTION OF GENOMIC DNA FROM BACTERIA BY CTAB METHOD

Bacteria from a saturated liquid culture were lysed and proteins removed by digestion with proteinase K. Cell wall debris, polysaccharides, and remaining proteins were removed by selective precipitation with CTAB. High-molecular-weight DNA was recovered from the resulting supernatant by Isopropanol precipitation. *A. hydrophila* strain was inoculated into a sterile 5-ml nutrient broth culture (NB) (to prepare NB, 10 g of peptone, 5g of yeast extract, 2.5 g of beef extract and 20 g sodium chloride were dissolved in one liter of distilled water and the pH was adjusted to 7.3) and grown over night at 28ºC. 1.5 ml of the culture was spun in a micro centrifuge for 2 min until a compact pellet formed. The supernatant was discarded. The pellet was resuspended in 567 µl TE buffer by repeated pipetting. 30 µl of 10% SDS and 3 µl of 20 mg/ml proteinase K were added to give a final concentration of 100 µg/ml proteinase K in 0.5% SDS. Mixed thoroughly and incubated for 1 hr at 37°C. 100 µl of 5 M NaCl was added and mixed thoroughly. 80 µl of CTAB/NaCl solution was added. Mixed thoroughly and incubated for 10 min at 65°C. An approximately equal volume (0.7 to 0.8 ml) of chloroform/isoamyl alcohol was added, mixed thoroughly, and spun for 4 to 5 min in a microcentrifuge. This extraction removed CTAB–protein/polysaccharide complexes. Aqueous supernatant was removed to a fresh microcentrifuge tube, leaving the interface behind. An equal volume of phenol/chloroform/isoamyl alcohol was added and extracted by spinning in a microcentrifuge for 5 min. The remaining CTAB precipitate was then removed in the phenol/chloroform extraction. The supernatant was transferred to a fresh tube. Isopropanol (0.6 ml) was added to precipitate the nucleic acids. The tube was shaken back and forth until a stringy white DNA precipitate became clearly visible. The pellet was transferred to a fresh tube

containing 70% ethanol by hooking it onto the end of a micropipette, which was previously heat-sealed and bent in a Bunsen flame. The DNA was washed with 70% ethanol to remove residual CTAB and respun for 5 min at room temperature to repellent it. The supernatant was carefully removed and the pellet briefly dried in a lyophilizer. The pellet was finally re-dissolved in 100 µl TE buffer.

Amplification of the 16S rRNA gene by PCR

The gene encoding the 16S ribosomal RNA (16S rRNA) gene of *A. hydrophila* was amplified by polymerase chain reaction (PCR) with the sequence of the primers and the corresponding annealing temperatures are given in the Table 1. Optimized PCR conditions are shown in Table 2. The amplification of the gene was performed in Eppendorf thermal cycler.

Table.1 Primers used for detection of 16S rRNA of *A. hydrophila*

Primer	Product size	Sequence (5'-3')	Annealing temperature
16S rRNAF 16S rRNAR	1035bp	TCG TTG GGT TGG GAT GTG TGT TAC CGC CGT GAA AGG	58°C

Table.2 Optimized PCR parameters for amplification of 16s rRNA of *A. hydrophila*

PCR parameters	Temperature (°C)	Minutes
i) Denaturation	95°C	5 minutes
ii) 30 cycles of Denaturation Annealing Elongation	 95°C 58°C 72°C	 40 seconds 40 seconds 50 seconds
iii) Final Extension	72°C	10 minutes

Collection of the Plant material

The leaves of *Lantana camara* plants were collected from Nanmangalam reserve forest Kanchipuram district 25 kms away from Chennai, Tamil Nadu, and India, 95° latitude 359°longitude.Seven hundred grams of *Lantana camara* leaves were collected and shade dried. After drying the weight of the dried leaves were recorded. Then, the leaves of *L. camara* were powdered using mortar pestle. The fine powder was further extracted with ethanol.

Extraction procedure

Fresh plant leaves was collected and shade dried (Fig. 1) for one month and the dried powder were extracted with 85% ethanol by cold percolation method giving a yield of about 30% (30 gms of extract from 100 grams of dried plant material) as per Venu *et al*., 2008.

Fig 1. Drying of *Lantana Camara* leaf

Qualitative analysis of phytochemical constituents in ethanolic extracts of *L. camara*

Tests for flavonoids, saponins, tannins, terpenoids and cardiac glycosides were performed by adopting the methods of Brinda *et al*., (1981); Kokate (1997); Harborne (1998).

Test for flavonoids:

To 1ml of stock solution in a test tube, few drops of dil. NaOH solution was added. Appearance of an intense yellow colour will indicate the presence of flavonoids after adding a few drop of dilute HCl.

Test for saponins:

1ml of stock solution was diluted to 20ml with distilled water and shaken continuously for 15 minutes. A foam layer on the top of the test tube will indicate the presence of saponins. **Test for tannins:**

To 3ml of stock solution in a test tube was diluted with 1 ml of chloroform and 1 ml of acetic anhydride was added and mixed well. To this mixture, 1 ml of sulphuric acid was added carefully by the side of test tube. Formation of green colour will indicate the presence of tannins.

Test for terpenoid (Salkowski test):

5 ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

Test for cardiac glycosides (Keller-Killani test):

5 ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxy sugar characteristic of cardiac glycosides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Antibacterial activity

Well diffusion method

Muller Hinton Agar medium was prepared and autoclaved as described above. The test organism was transferred from the stock into a glass test tube containing 5 ml of sterile nutrient broth with the help of a wire loop. The inoculated broth was incubated at 28ºC for 10-12 hrs to obtain moderate turbidity.

The agar well diffusion method (Perez *et al.,* 1990) was followed. A sterile cotton swab was dipped into the broth culture inoculums. The cotton swab was then rotated pressing against the inside wall of the tube, above the fluid level to remove excess inoculums *A. hydrophila was,* inoculated. The agar surface of the plate was inoculated by swabbing three times, turning the plate by 60º angle in between swabbing. The lid of the Petri dish was replaced and the plate was kept at room temperature for 5 to 10 minutes to dry the inoculums. Five wells of 8 mm diameter were punched into the agar on plate using a sterile well cutter. In each well, 100µl of (different concentration of 2.5, 5, 7.5, 10 mg/ml) plant extract was added. Into the other well, 100µl saline was added. The solutions were allowed to diffuse for 2 hrs. The plates were incubated at 28ºC for 24-48 hrs. The antibacterial activity was evaluated by measuring the zone of inhibition around the well.

Minimum Inhibitory Concentrations (MIC) Ciprofloxin and Ethanolic leaf extract against *A. hydrophila*

The MIC of Ethanolic leaf extract of *Lantana camara* against *A. hydrophila* was also determined by the tube dilution technique. The nutrient broth was prepared and autoclaved. The nutrient broth was allowed to cool and varying concentrations of leaf extract was (11.7, 23.4, 46.7, 93.75, 187.5, 375, 750, 1000 and 1250mg/ml) were added to the broth before pouring into the test tubes and allowed to cool. The MIC of positive control (Ciprofloxin) against *A. hydrophila* is followed as per the above procedure.

A. hydrophila was inoculated into the tubes and tubes were incubated at 28ºC for 24 hrs. After 24 hrs the tubes were examined for bacterial growth in the broth. The growth of the organism was measured by the method described earlier.

Experimental Animal collection

Mrigal fingerlings, (average size and weight 10.7±1.0cm and 8.65±1.5gm) were obtained from Guruswamy fish farm, Pondicherry. The fishes were screened for any pathological symptoms and healthy fingerlings, only were selected for the experiment. Uniform size group fingerlings were selected and transfer to 3 liter capacity of glass tanks. Fishes were acclimatized for four days to get rid of handling stress. The experimental tanks where provided with aerations. The fish were subsequently fed with commercial fish pellet feed (Fig. 2).

The metabolic waste and fecal matters where siphoned-out with about 80% water exchanged with filtered fresh water. On the day of treatment (plant extract) and challenging study only the fecal matter was removed and no water exchange was done. The experiment was conducted under the normal light regime (light and dark 12/12 h). The water quality parameters of experimental tanks where periodically monitored in the standard range of Water temperature 29 ± 1ºC, Salinity 1.4 ppt, pH 6.0 ± 0.5, Dissolved Oxygen 5.5 - 5.8 ppm, NH_3 0.5-0.8 ppm, Nitrate 0.4-0.6 ppm.

Pathogenicity experimental set up

Infection through Intramuscular injection

The healthy Mrigal fingerlings at the rate of five fish per tank were maintained in 3- l capacity glass tanks. The water was provided with good aeration and it was changed daily. The animals were fed with artificial fish feed. The healthy fish, 60 µl of bacterial suspension was injected intramuscularly using 1-ml insulin syringes. Control fish received only sterile saline. The experiment was conducted in triplicates. Animals were checked twice daily for clinical signs of diseases and mortality. Dead animals were removed.

Fig.2. Experimental tank setup

Plant Extract Treatment

L. camara leaf extract was injected through intramuscularly; the concentration of plant extract was 60 µg in 8 gms of fish. A control group of fishes were maintained under the same conditions without Treatment. The positive control also maintained. These experiments were conducted in triplicates.

Bacterial Challenge

The untreated and treated Mrigal fingerlings were challenged with Bacteria through intramuscular injection methods the concentration of bacterial suspension was 60 µl. The animals (untreated, treated and normal fish) were grouped into 3 groups,

namely group I for positive control, group II Treated or challenged and group III for Normal control. Three replicates were used in each batch. The bacteria inoculums were prepared as per the protocol described elsewhere in this section. The Mrigal fingerlings were placed 5 nos in 3 litres of water in tanks containing freshwater with continuous aeration. The tanks were covered to prevent contamination.

After immunization, the fish were challenged with *A. hydrophila* cells by intramuscular injection. Control fish were also injected with *A. hydrophila*. The cumulative percent mortality (CPM) and RPS were calculated. The RPS was calculated using the following formula.

$$RPS = \left\{1 - \frac{\%\,Mortality\,in\,Treated\ \ fish}{\%\,Mortality\,in\,\text{control fish}}\right\} X\ 100$$

Histopathological investigation of Mrigal fingerlings

Histopathology

For histological investigation, organs such as kidney, liver and gills, were dissected out from experimentally infected Mrigal fingerlings. The histology of these organs in normal fish was also studied. The organs were fixed in 10% neutral buffered formalin (NBF) fixative. Later, the organs such kidney, liver and gill were cut and stored in screw cap bottles containing the fixative. For 10% neutral buffered formalin (NBF) fixative, the initial fixation time was 48 hours; thereafter the materials were transferred to 70% alcohol and stored.

Processing of tissue and staining

For cutting sections of different tissues in paraffin, dehydration and clearing of the tissues were carried out at room temperature. The tissues were first washed in two changes of 70% alcohol for one hour each, dehydrated for two hours in two changes of 70% alcohol for one hour each, further dehydrated for one hour each in two changes of 80% alcohol, graded twice in 95% alcohol and in absolute alcohol, cleared through a mixture of absolute alcohol and chloroform (1:1 v/v) and then passed twice in pure chloroform for one hour each. Chloroform was preferred over xylene as the former did not cause the tissue to become hard and brittle. The tissues, after clearing, were left in a mixture of chloroform and paraffin wax (approximately 1:1) at room temperature overnight. Before embedding, the tissues were impregnated in three changes of paraffin wax with ceresin of 58 to 60°C melting point for one hour each. The transverse sections were cut at 5 to 7 µm thickness using a manual rotatory microtome. After deparaffinishing in xylene, the sections were hydrated through graded series of alcohol up to 70% and stained with Harris alum hematoxylin and counterstained with 1% alcoholic eosin (Preece, 1972). Applying the routine procedure, stained sections were dehydrated through the graded series of alcohol and mounted with glass cover slip in DPX through xylene.

Light microscopy and photomicrography

The histology sections were studied using Carl Zeiss binocular compound microscope. Cellular measurements were taken with Carl Zeiss microscope fitted with a calibrated ocular micrometer scale having accuracy up to 10 µm. Photomicrographs were taken with digital camera (Nikon) attached to Carl Zeiss microscope with projection eyepiece 10 X and objectives 10, 20, 40 and 100 X. The magnification of the enlarged prints was calculated with ocular and stage micrometer.

Physico-Chemical parameters

Physico-Chemical parameters of the experimental and control water samples such as pH, Temperature, Salinity and Dissolved oxygen (DO) were measured. The dissolved oxygen was estimated by the Winkler method. Ammonia was estimated by the Nesslerization Method and Nitrate was estimated by the UV Spectrophotometer method.

Dissolved Oxygen (Winkler's method)

Collected sample in a BOD bottle using D.O sampler. Added 1 ml $MnSO_4$ followed by 1 ml of alkali-iodide-azide reagent to a sample collected in 250 to 300 ml bottle up to the brim. The tip of the pipette was below the liquid level while adding these reagents and stoppered immediately. Pipettes were rinsed before putting them into the reagent bottles. Mixed well by inverting the bottle 2-3 times and allowed the precipitate to settle leaving 150 ml clear supernatant. The precipitate was white if the sample was devoid of oxygen, and became increasingly brown with rising oxygen content. At this stage, added 1 ml conc. H_2SO_4. Replaced the stopper and mixed well till precipitate went into solution. 50 ml of this solution was taken in a conical flask and titrated against 0.025 N $Na_2S_2O_3$ solution using starch (2 drops) as an indicator. The end point was the disappearance of blue colour.

Estimation of Ammonia by Nesslerization method

100mL of sample was taken. 1 ml ZnSO4 solution and 0.4 or 0.5 ml NaOH was added to obtain the pH of 10.5. It was allowed to settle and filtered the supernatant through Whatman filter paper No 42. Suitable aliquot of sample was taken. 3 drops of Rochelle salt solution was added with 1 drop of EDTA and mixed well. 3mL of Nessler's reagent was added. EDTA is used or 1mL if Rochelle salt solution. It was making up to 100mL. It was mixed well and percentage of transmission was observed after 10 minutes at 410nm using a blank prepared in the same way by taking distilled water instead of sample.

Agarose gel electrophoresis

Agarose gels ranging from 0.7% - 1.2% were used for separation of DNA depending on the size of the product or fragments to be separated. Gels were prepared with TEB (1X) buffer. The PCR amplified product mixed in 1/3 volume of loading dye was loaded along with the DNA marker. Electrophoresis was performed at 10 V/cm until the dye reached the end of the gel. The gel was then gently removed and stained in ethidium bromide staining solution for 15 minutes. Destaining was done in distilled water or TEB solution with gentle shaking for 15-30 minutes. The electrophoresis DNA gel was photographed under UV light source for a permanent record of the result.

Preparing finfish samples for histology

Fixing specimens for histology involves the handling of potentially dangerous chemical products. Always wear gloves and goggles (or protective glasses), when handling fixatives (and fixative components) and throughout the fixation process.

Sampling of fish:

Only live, moribund or recently dead (less than ½ hour to 1 hour) specimens with apparent signs of disease should be fixed for histology examination. Do not sample that already dead fish.

Sample fixation:

Fish samples were anaesthetized or chilled and then immediately fixed with appropriate fixative (10% NBF). Fish fry-those measuring less than 3 cm can be injected fixative into abdominal cavity and placed whole fishes in fixative. Fish that are larger than 3 cm in body length open up the body cavity to allow fixative to penetrate into the deeper tissues. Fish that are larger than 10 cm open the body cavity and dissect the target organs like Brain, eye, Liver, Spleen, Kidney, Heart and Gills immediately placed into fixative. It is recommended that at least ten volumes of fixative for each volume of tissue samples (ratio of 1part tissue to at least 9 parts formalin). Samples are allowed to fix for at least 24-48 hours before processing. Each sample was carefully labeled by pencil.

Microtome sectioning

Sectioning an embedded tissue sample is the step necessary to produce sufficiently thin slices of sample that the details of the microstructure of cells/tissues can be clearly observed using microscopic techniques.

Microtome is the mechanical device used for cutting uniform sections of tissue of appropriate thickness by trimming the surface of the paraffin tissue block. Section thickness is set to the appropriate level of 4-5 microns for routine purposes. The sliced paraffin block becomes a fragile ribbon made of wax. It is gently placed into warm water bath and picked it up on adhesive coated slides.

Haemotoxylene and Eosine staining

Routine histological preparations typically employ a standard stain; the Bennett modification of Mayer's hematoxylin and phloxine/eosin (Sugita *et al.*, 1994) stain, which provides an excellent routine H&E stain for shrimp tissues with this method cell nuclei are stained blue and cytoplasm and many extra-cellular components in shades of pink.

Mounting and Observation

Resinous mounting media (DPX) is routinely used for mounting agent. After H&E staining and clearing off the slides, add a drop of mount ant on the cover slip and slides are inverted on to the cover slip. Care should be taken to avoid air bubbles. Do not press the slide or cover slip, it can damage the section.

Results

Morphological characters of the isolates

Colonial and cell morphology

On the Soybean Casein Digest Agar plates showed that the colonies were appears circular, 1-3 mm in diameter, convex, smooth, moist, opaque, and greyish-white colonies with uniform shape (Fig 3.a). On selective TCBS agar medium showed that colonies were circular and yellow colonies in (Fig. 3.b).

Gram staining

Bacteria isolated from colony morphology in SCDA agar media were presumptively identified as *Aeromonas hydrophila;* they are Gram- negative, curved rod shape, motile bacteria in (Fig. 4).

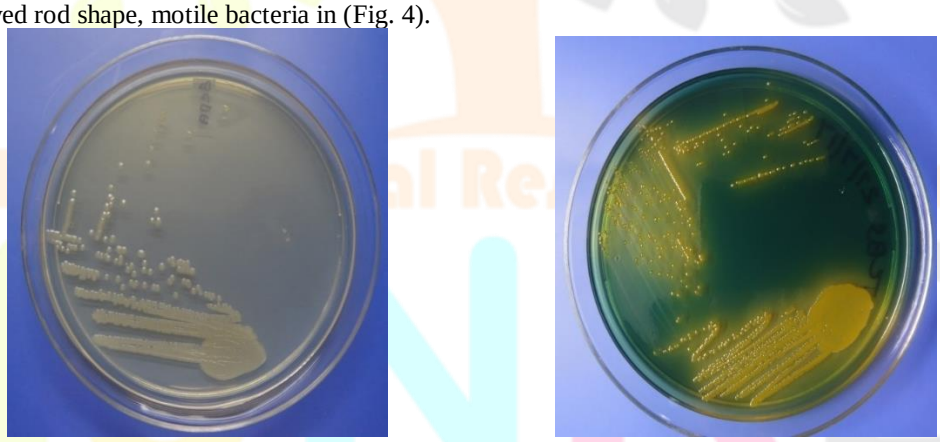


Fig.3: Morphology of *Aeromonas hydrophila* (a) in SCDA (b) in TCBS

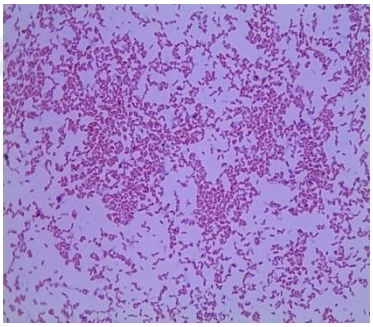


Fig. 4. Gram Staining of *Aeromonas hydrophila*

Biochemical Analysis

As for the biochemical analysis is concerned the *A. hydrophila* was produced the oxidase and catalase enzymes and it was a motile bacterium.

Table. 3. Biochemical Analysis of *Aeromonas hydrophila*

S. No	Test Analysis	Results
1.	Motility	+
2.	Catalase	+
3.	Oxidase	+
4.	Haemolysis	+

Hemolysis Test

The hemolysis test showed that the *A. hydrophila* exhibit β-hemolysis that is complete lysis of blood cells in agar media, shown in Fig. 5.

Fig. 5. Haemolysis test of *Aeromonas hydrophila*

PCR documentation:

A. hydrophila was confirmed by PCR analysis also using primers specific to bacteria and results shown in Fig.6 the results showed the appearance of prominent band of PCR amplified product (1035 bp) for postive samples and no corresponding band was observed in Negative control.

Zone of inhibition

L. camara plant species were investigated to evaluate their antibacterial activity against aquatic bacteria strains of Gram-negative bacteria *Aeromonas hydrophila* using well diffusion method. Evaluation of antibacterial activity of this plant extract was represented in Table 4 and in Fig. 7a. and 7b. The Ethanolic leaf extract is loaded in each plate showed inhibition of bacterial growth against *A. hydrophila*. The plant extract exhibited inhibition zones ranging from 6 mm to 16 mm diameter, with the most net worthy results shown by *A. hydrophila*. Ciprofloxin 10 µg disk (16 mm) showed a resistant result. The diameters included the 6 mm filter paper disk. The Ethanolic extract prepared from the *L. camara* leaf demonstrated inhibition zones greater than 16 mm in diameter. Results of antimicrobial activity of the *L. camara* extract can suggested that *A. hydrophila* were the most susceptible strain to the extracted plant respectively. Hence, experiments were conducted to determine their minimal inhibitory concentration (MIC) against the most susceptible bacterial strain (*A. hydrophila*).

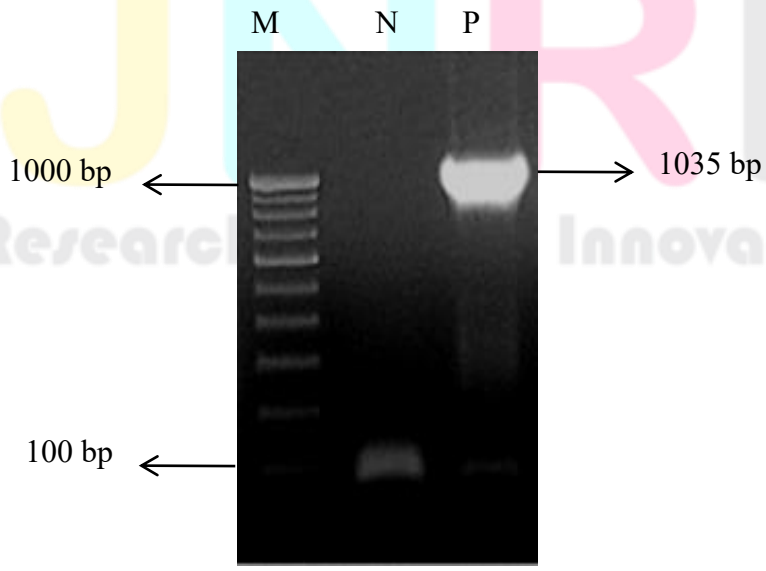


Fig. 6. PCR confirmation of *Aeromonas hydro*phila used in the present study using specific primer set. Lane-M 100 bp DNA marker; Lane N- Negative control, Lane P- Inoculums of *Aeromonas hydrophila*

Table. 4. Mean zones of inhibition (mm) of different concentrations *Lantana camara* extract against *Aeromonas hydrophila*

S. No	Name of the Sample	Against *Aeromonas hydrophila*					
		Positive Ciprofloxacin	100 µg	250 µg	500 µg	750 µg	1000 µg
1.	Plant Extract - Water	24 mm	-	-	6 mm	13 mm	15 mm
2.	Plant Extract - DMSO	24 mm	-	-	7 mm	14 mm	16 mm

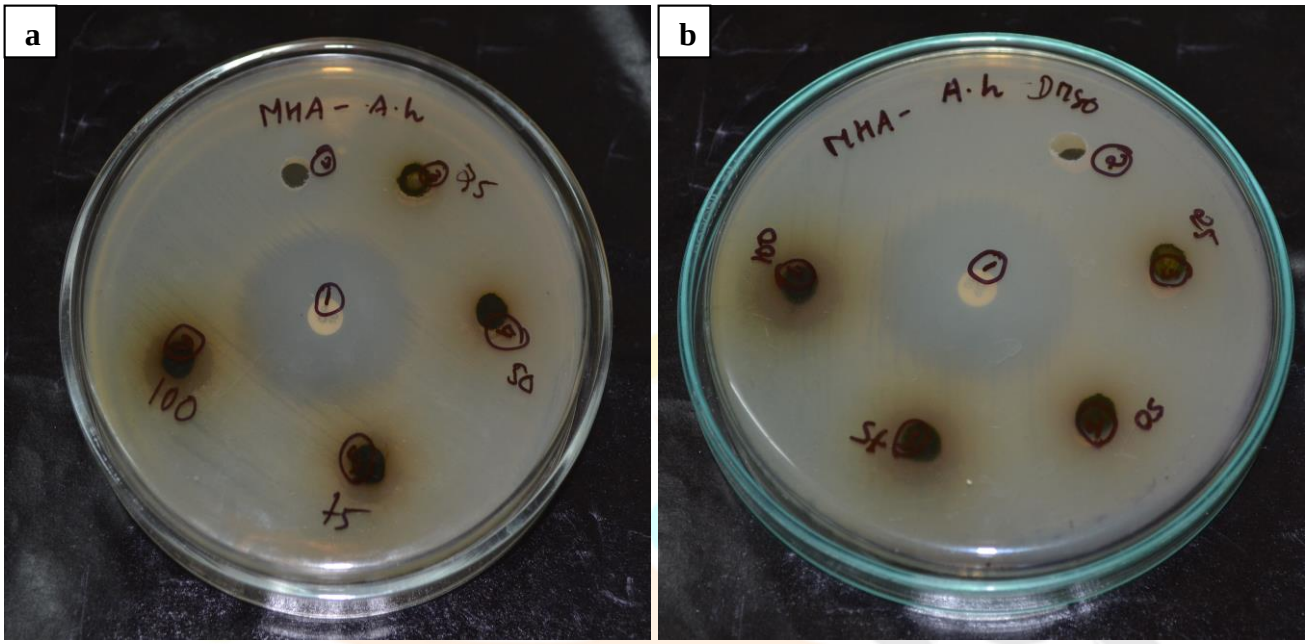


Fig. 7. Antibacterial activity of different concentration of *L. camara* extract against *Aeromonas hydrophila* by well diffusion method
(a) Plant extract diluted in water and (b) Plant extract diluted in DMSO.

Minimum Inhibitory Concentration (MIC)

The MIC test for the ethanol leaf Extract of *L. camara* against *A. hydrophila* (Table. 5 and Fig. 8a and 8b) shown that the bacterial growth was noticed at the 0.375 mg concentration of plant extract. Hence, the concentration of plant extract 0.500 mg *of L. camara* has the minimum inhibitory concentration against *A. hydrophila.*

Table. 5. Minimum Inhibitory Concentrations of different concentrations plant extract (*Lantana camara*) against *Aeromonas hydrophila*

S. No	Name of the Sample	Against *Aeromonas hydrophila*								
		Positive Media alone	Different concentration of Plant extract µg/ml							
			11.7	23.4	46.7	93.75	187.5	375	500	750	1000
1.	Plant Extract – Water	+++	+++	+++	++	++	++	+	-	-	-
2.	Plant Extract – DMSO	+++	+++	+++	++	++	++	+	-	-	-

+++ = High growth; ++ = moderate growth; + = poor growth; - = no growth

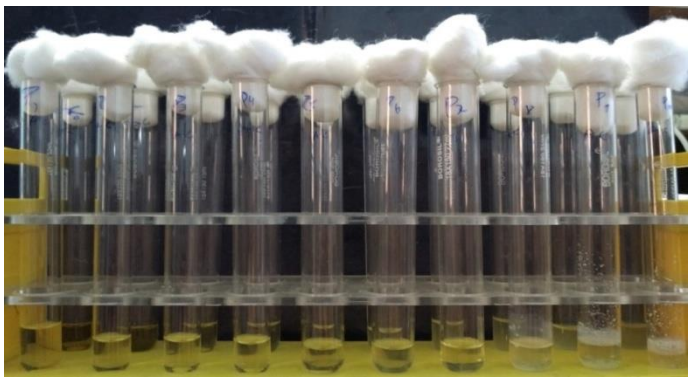


Fig. 8. (a) Minimum inhibitory concentration of Positive Control (Ciprofloxin) against *A. hydrophila*

Fig.8 b Minimum inhibitory concentration of *L. camara* against *A. hydrophila*

Phytochemical Analysis

The phytochemical present in ethanolic leaf extract of *L. Camara* were represented in Table. 6 and Fig.11. Saponins and Tannins were present in ethanolic extract sample of leaves, whereas Flavonoids, Terpenoids and Cardiac glycosides was absent in ethanol extract.

Pathogenicity

Aeromonas hydrophila challenged *Mrigal* fingerlings exhibited sluggishness and abnormal behaviour like wandering around the corners, resting at the bottom and vertical swimming. The clinical signs of infected *Mrigal* fingerlings include lesions on the body surface, rough skin and scale loss was found (Fig.12). The ulcerative lesions are seen most often with reddish colouration on the opercular surface. These signs were observed in infected *Mrigal* fingerlings in the experimental tank. Slight body fouling was also noted in dead fish. The mortality ranged from 80-90% during pathogenicity study.

Cytotoxicity of *Lantana camara* extracts on *Cirrhinus cirrhosus* Kidney Cell Line:

The plant extract at higher dilution of above 750 µg/ml developed CPE after 24 hr exposure represented in Fig. 9.

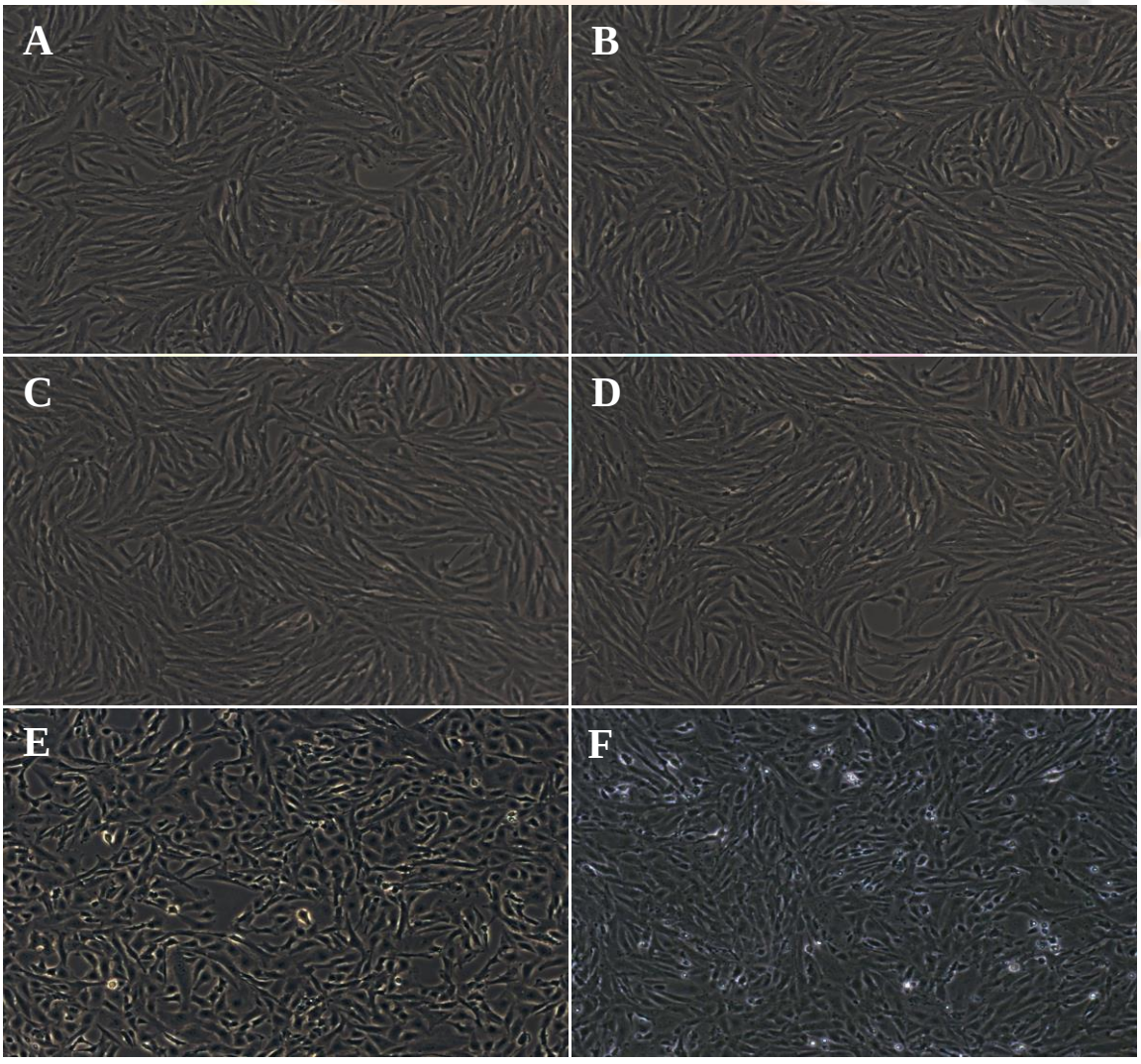


Fig. 9. Morphological alterations were observed in *Cirrhinus cirrhosus* Kidney cell line exposed to different concentration of *Lantana camara* extract (A) control cell line, (B) 100 µg/ml (C) 250 µg/ml (D) 500 µg/ml (E) 750 µg/m land (F) 1000 µg/ml (100 X magnification).

***In vitro* Cytotoxicity**

In vitro Cytotoxicity of *Lantana camara* extract to Kidney cell line of *Cirrhinus cirrhosus* after 24 hrs exposure has shown in Fig. 10.

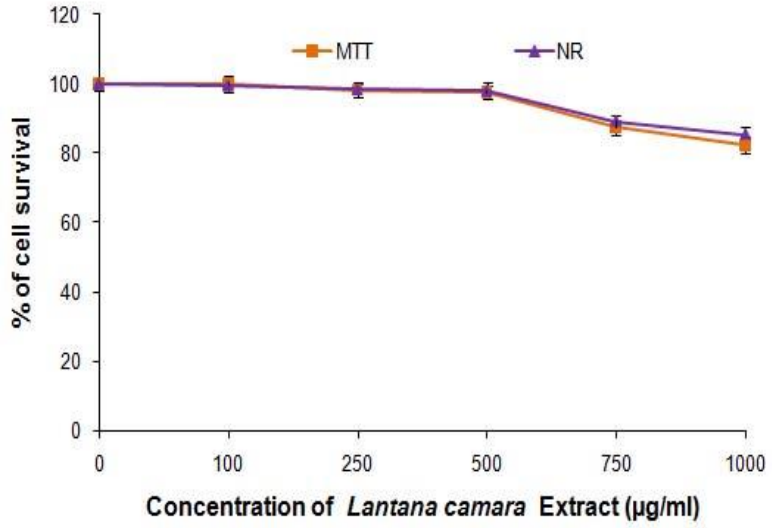


Fig. 10. *In vitro* Cytotoxicity of *Lantana camara* extract to Kidney cell line of *Cirrhinus cirrhosus* after 24 hrs exposure by MTT and NR uptake assay. The individual data points are expressed as the arithmetic mean percentage of control (mean ± SE) (n = 8 replicate).

Table. 6. Phytochemical analysis of ethanolic leaf extract of *L. camara*

S. No	Phytochemical Test	Result
1	Flavanoids	-
2	Saponins	+
3	Tannins	+
4	Terpenoid	-
5	Cardiac glycosides	-

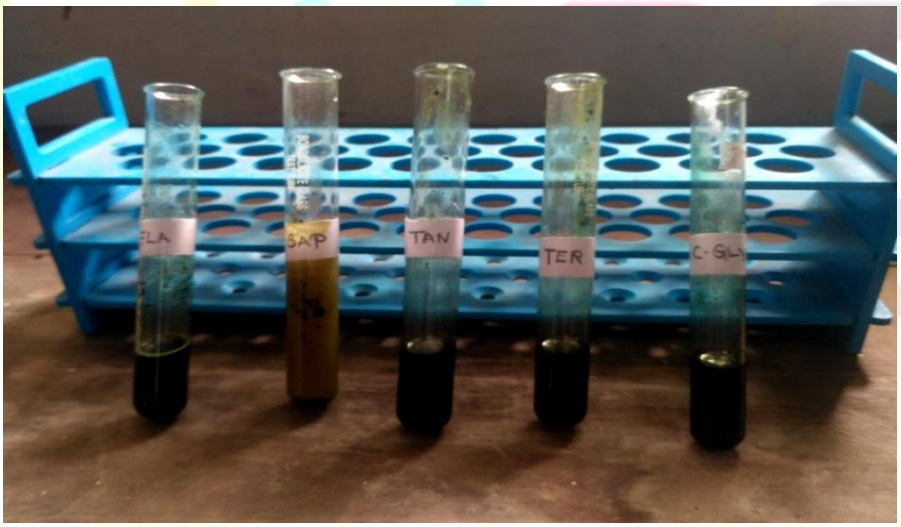


Fig.11. Phytochemical analysis of Flavonoids, Saponin, Tannin, Terpenoids and Cardiac glycosides.

Fig. 12. Infected Mrigal fingerlings fish

The *Mrigal* fingerlings were exposed to the bacteria through cohabitation fingerlings of 8.65±0.5gm size was used for evaluation. Observation was made for a period of 10 days of post infection (dpi) indicates that the chronic susceptibility of the fingerlings and effect was observed from 6 dpi. The cumulative mortality was chronic and maximum was observed (80%) on 6 dpi (Fig. 13).

Plant treated and challenging study

Mrigal fingerlings were treated standard protocol with the plant extract by intramuscular injection. The Treated fishes were challenged with bacteria by standard protocol to study the efficacy of ethanol extract to protect the fingerlings from *A. hydrophila* bacterial infection the result are shown in Fig. 14. The Cumulative mortality in fingerlings was 80% in non-treated fish at 6 days of post-infection with the bacteria; in this case of fishes the mortality was30% in treated fish and RPS value was calculated and shown in Table. 7. The RPS value was 63%. The result indicates that treated plant extract can be used through the intramuscular injection for Mrigal fingerlings to protect against bacterial infection. Plant treated and control fish by intra-muscular injection challenged with *A. hydrophila* at 4 days of post treated and observed for 10 days

Histopathological Studies

For further infection of the bacteria on the fishes were exposed to bacterial infection, histopathological investigation of the Kidney, Gills and liver was carryout on experimental groups confirmed by light microscope.

Histopathology Results (*Mrigal* fingerlings organs) Against *A. hydrophila*

On 6-day post-injection (dpi), 80% of the challenged *Mrigal* fingerlings died. The histopathological changes noted in the tissues of *Mrigal* fingerlings challenged by *A. hydrophila* are depicted those of kidney, gill and liver.

Gill

The Gills of control fish showed normal organization with lamellar epithelium, infected gill showed bronchitis at lamellae and hyperplasia, treated gill showed normal gill lamellae. The gills revealed mild proliferation of the covering epithelium particularly at the base of the secondary lamellae and haemocytic infiltration and congestion (Fig. 15).

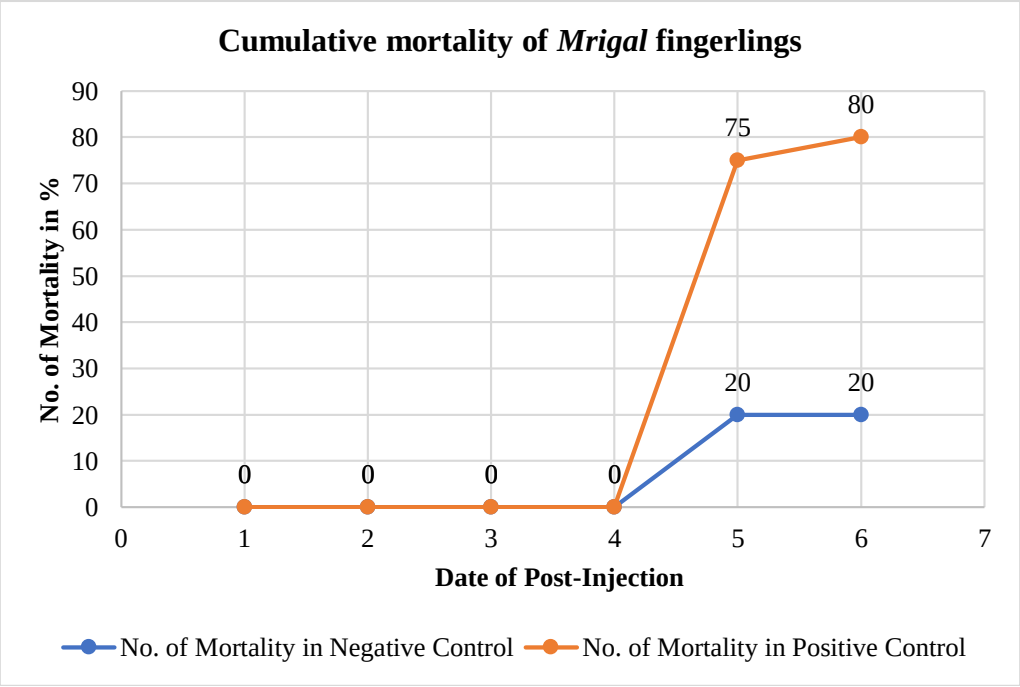


Fig. 13. Cumulative mortality of *Mrigal* fingerlings after infection with *A. hydrophila* through intra-muscular injection route

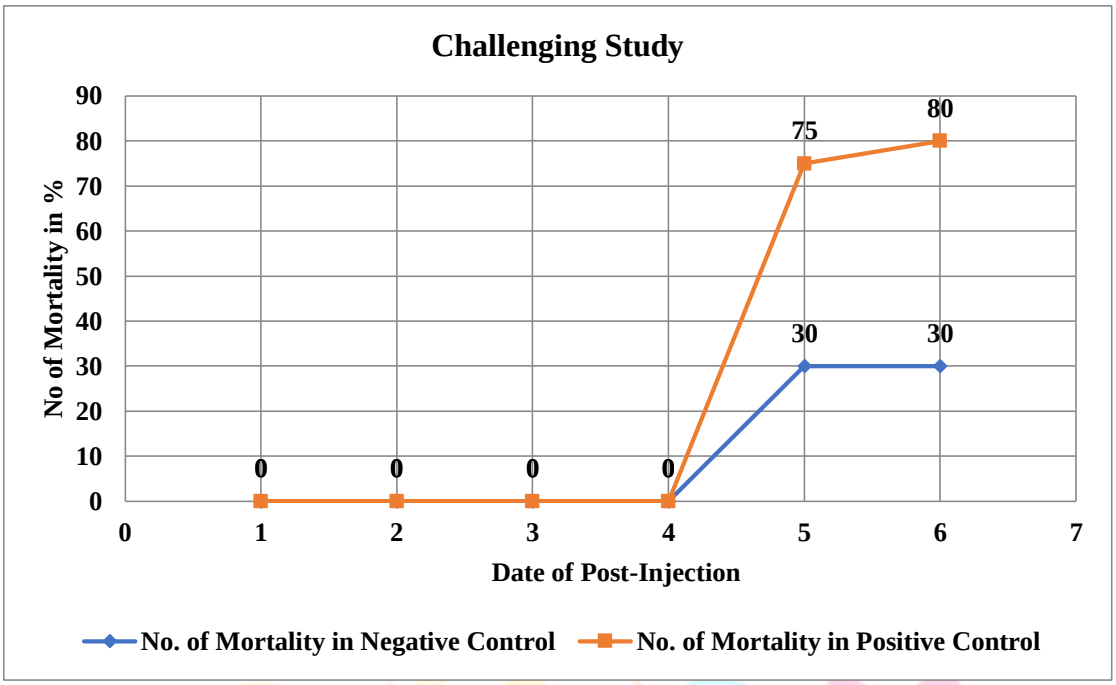


Fig. 14. Mortality of *Mrigal* fingerlings with *A. hydrophila*.

Table. 7. Relative percentage survival (RPS) of treated and non-treated fishes

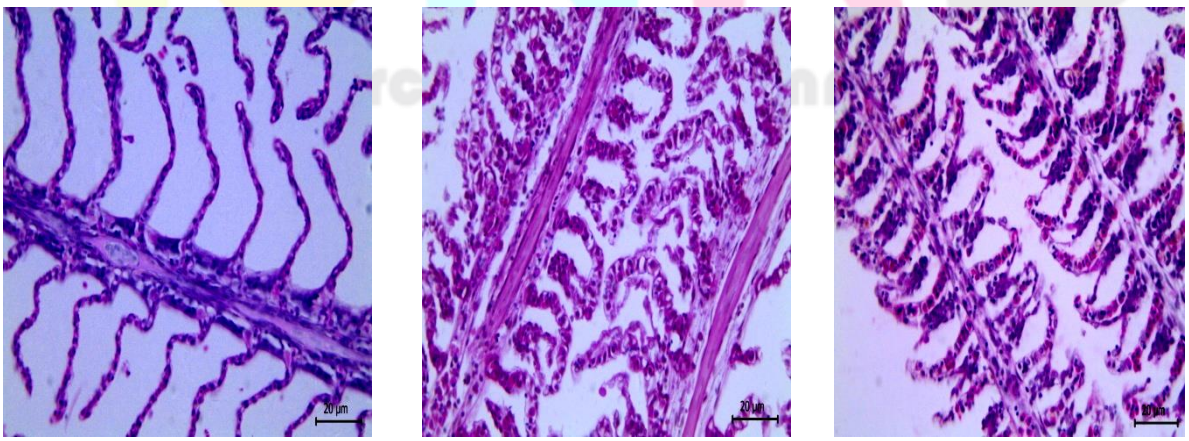
Fish immersed with *Aeromonas hydrophila*	**Cumulative percentage mortality (Dead fish/total immersed fish)**	**Relative percentage survival (RPS)**
Negative control	20.00% (2/10)	-
Positive control	80.00% (8/10)	-
Treated fish	30.00% (3/10)	63%

Kidney

The Kidney of control and treated *Mrigal* showed normal organization and kidney tubules, hematopoietic tissue and glomeruli were arranged in normal fashion. The kidney of the experimentally infected *Mrigal* with *A. hydrophila* bacteria developed hematopoietic atrophy, degeneration of hyaline droplets with vacuolations and glomerular degeneration (Fig. 16).

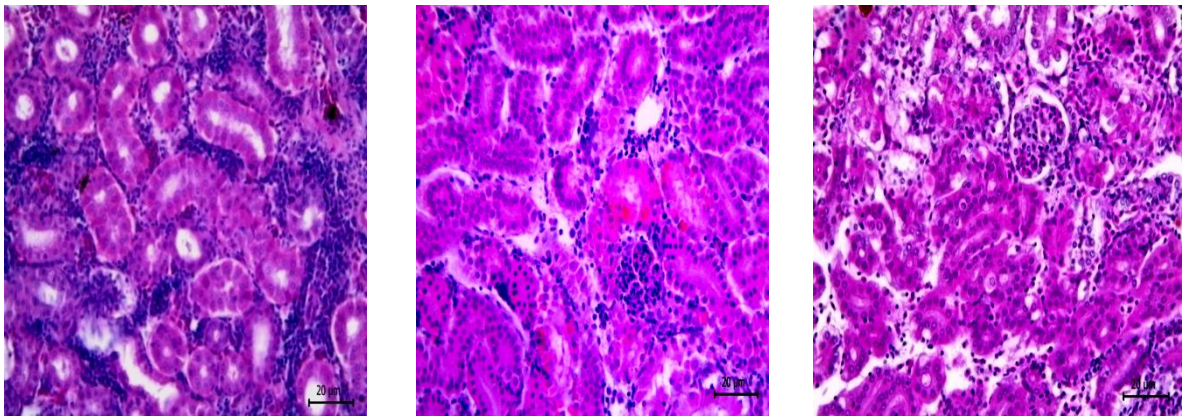
Liver

The Liver of control *Mrigal* contained all the hepatocytes were arranged in normal texture. Hepatocytes of the experimentally infected *Mrigal* fish were evidenced by uncoordination of the tissue. Bacterial interference in the hepatocytes caused vacuolation and haemorrhage, treated *Mrigal* showed normal structure with mild haemocytic inflammation (Fig. 17).



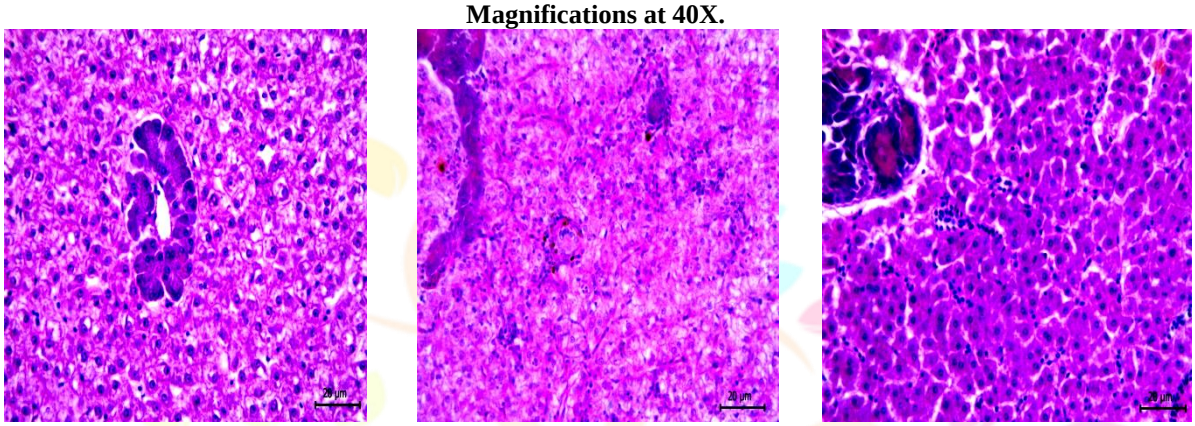
(a) Normal Gill **(b) Negative control Gill** **(c) Treated Gil**

Fig. 15. Histology of *Mrigal* Gill stained with Haemotoxylene and Eosine Magnifications at 40X.



(a) Normal Kidney (b) Negative control Kidney (c) Treated Kidney

Fig. 16. Histology of *Mrigal* Kidney stained with Haemotoxylene and Eosine Magnifications at 40X.



(a) Normal Liver (b) Negative control Liver (c) Treated Liver

Fig. 17. Histology of *Mrigal* Liver stained with Haemotoxylene and Eosine Magnifications at 40X

Discussion

Indian aquaculture has demonstrated a six-and-a-half-fold growth over the last two decades, with freshwater aquaculture contributing over 95% of the total aquaculture production. Carps are the mainstay of aquaculture production in the country, which is supported by strong traditional knowledge base and scientific inputs in various aspects of management. The ability of a pathogen to locate, attach to, and subsequently infect a susceptible host is a primary step in the development of disease. Consequently, factors produced by motile aeromonads, which can facilitate contagion, are important elements of bacterial virulence. In fact, motile aeromonads that have been taken from lesions on diseased fish have been shown to have a greater chemo tactic response to skin mucus than isolates that were obtained as free-living organisms from pond water.

The plant possesses numerous biologically active compounds which could serve as potential source of vegetable drugs in herbal medicine (Chukwuma *et al.*, 2010). *Lantana camara* is a common available ornamental plant, which has many biological active components (Ferrav *et al.*, 1995). The antibacterial efficacy of the plant extract was quantitatively assessed on the basis of zone of inhibition. All the parts studied in the investigation exhibited varying degree of inhibitory growth effect against the selected bacterial strains. Among all the parts (root, stem, leaves, flower, and fruit) of *Lantana camara*, the stem was found to be more active against livestock pathogens. This is due to the presence of one or more bioactive compounds such as flavonoids, proteins, alkaloids, tannins etc, present in the particular parts. Plant which are rich in tannins have antibacterial potential due to their character that allows them to react with proteins to form stable water-soluble compound thereby killing the bacteria directly by damaging the cell membrane (Franca *et al.*, 1996). The basis of varying degree of sensitivity of test organisms of bacteria may be due to the intrinsic tolerance of microorganisms and the nature and combinations of phytocompounds present in the extract.

The results of the present study the Ethanolic leaf extract is loaded in each plate showed inhibition of bacterial growth against *A. hydrophila*. The plant extract exhibited inhibition zones ranging from 6 mm to 16 mm diameter, with the most noteworthy results shown by *A. hydrophila*. Ciprofloxin 10 µg disk (16 mm) showed a resistant result. The diameters included the 6 mm filter paper disk. The Ethanolic extract prepared from the *L. camara* leaf demonstrated inhibition zones greater than 16 mm in diameter

Results of antimicrobial activity of the *L. camara* extract can suggested that *A. hydrophila* were the most susceptible strain to the extracted plant respectively. Hence, experiments were conducted to determine their minimal inhibitory concentration (MIC) against the most susceptible bacterial strain (*A. hydrophila*). The MIC test for the ethanol leaf Extract of *L. camara* against *A. hydrophila* (Table 5.3) shows that the bacterial growth was noticed at the 0.375 mg concentration of plant extract. Hence, the concentration of plant extract 0.500 mg *of L. camara* is the minimum inhibitory concentration against *A. hydrophila.*

Similar results with other plant extract against Gram positive and Gram-negative bacteria were reported by others (Grover *et al.*, 1962). 20µl acetone extract of other plants like *Glycyrrhiza glabra, Cinnamomum cassia, Juniperus oxyudrus* elicited maximum antibacterial activity against *B. brevis, B. cereus, B. megaterium, B. subtilis, P. aeruginosa and S. aureus* (Leite *et al.*, 2006). The antimicrobial activity may be due to the presence of triterpene secondary metabolite in the extract. Similarly, Borrel *et al.*, 1997 have reported a bioactive triterpene-22 beta acetoxylantic acid and other triterpene which showed antimicrobial activity against *Staphylococcus aureus* and *Salmonella typhii*. Antibacterial activity of different plant extract on phytopathogenic *Xanthomonas campestris* pathovars was studied and reported by other workers (Mazumder *et al.*, 2004). PCR was performed to identify the bacterial isolate at molecular level using primers specific to 16S rRNA gene of *A. hydrophila*. Borrell *et al.*, 1997 reported that a good and rapid way of assessing the identities of all known species of *Aeromonas* is by computer analysis of the published 16sRNA

gene. PCR technique is widely used as an effective tool to detect bacteria. The primer pair was designed on the basis of the published nucleotide sequence of 16S rRNA gene of *A. hydrophila*. The primer pair specific to 16S rRNA gene of *A. hydrophila* amplified a DNA fragment of approximately 257 bp in the DNA prepared from *A. hydrophila* isolated from infected fish. Khan and Cerniglia (1997) have developed a PCR based detection method to detect the *A. hydrophila* using 16S rRNA.

A wide range of putative virulence factors have been detected and studied in several *Aeromonas* spp. (Albert *et al.*, 2000; Gonzalez- Serrano *et al.*, 2002; Kingombe *et al.*, 1999; Sechi *et al.*, 2003) they play a pivotal role in the establishment of infection. Indeed, several studies have reported the detection and characterization of virulence factors in *Aeromonas* spp. isolated from freshwater fish, Mrigal *(Cirrhinus cirrhosus)*, humans, meat-producing animals and potable water (Albert *et al.*, 2000; Escarpulli *et al.*, 2003; Granum *et al.*, 1998; Gonzalez-Serrano *et al.*, 2002; Huizinga *et al.*, 1979; Majeed *et al.*, 1989). There are some reports that high mortality of fish is associated with the presence of *A. hydrophila* (Munro *et al.*, 1993; Ringo and Vadstein, 1998). Munro *et al.*, (1993) noted low survival of fish larvae associated with high proportion of *A. hydrophila*. High colonization of *A. hydrophila* in the gut of turbot fish larvae caused 100% mortality in fish larvae (Ringo and Vadstein, 1998). There are many reports on the isolation of *A. hydrophila* from other fishes like carp, gold fish, catfish, mullet and tilapia (Sugita *et al.*, 1994, 1995). The results of the present study the chronic susceptibility of the fingerlings and effect was observed from 6 dpi. The cumulative mortality was chronic and maximum was observed (80%) on 6 dpi. The clinical signs of infected Mrigal fingerlings include lesions on the body surface, rough skin and scale loss was found. The ulcerative lesions are seen most often with reddish coloration on the opercular surface. These signs were observed in infected Mrigal *fingerlings* in the experimental tank. Slight body fouling was also noted in dead fish. The mortality ranged from 80-90% during pathogenicity study.

The large-scale settings of aquatic animal husbandry have resulted in an increased antibiotic resistance in bacteria potentially pathogenic to fish and related environment (Smith *et al*., 1994; Alderman and Hastings, 1998; Petersen *et al*., 2002; Alcaide *et al*., 2005; Cabello, 2006). The continuous use of antimicrobial agents in aquaculture has resulted in more resistant bacterial strains in the aquatic environment. Continuous use of synthetic antibiotics reveals the threats to consumers and non-target organism in the environment (Muniruzzaman and Chowdhury, 2004; Abutbul *et al*., 2005). Treatment of bacterial diseases with various herbs has been safely employed widely in organic agriculture, veterinary and human medicine (Direkbusarakom, 2004). Since ancient times, medicinal plants have been used for the treatment of common infectious diseases (Rios and Recio., 2005) and treatments with plants having antibacterial activity are a potentially beneficial alternative in aquaculture (Abutbul *et al*., 2005). Medicinal plants as the alternative agents are effective to treat the infectious diseases and mitigate many of the side effects that are associated with synthetic antimicrobials (Punitha *et al*., 2008).

Thomas *et al.*, 2012 and Azad *et al.*, 2001, described that challenged tilapia showed haemorrhages at the site of infection and experienced 90% mortalities. The main visceral organs, viz., kidney, liver, gill and spleen were affected in the infected fish, thus revealing the fact that *A. caviae* was able to infect many visceral organs similar to *Aeromonas caviae* like bacterium and *A. hydrophila*. Systemic infections are generally characterized by diffused necrosis in several internal organs; primarily the liver and kidney are the target organs of an acute septicemia. These organs when attacked by bacterial toxins cause acute haemorrhage and necrosis of vital organs and lose their structural integrity (Huizinga *et al*., 1979). Anwesha Roy, *et al.*, 2018 described in control and plant treated fish; the normal structure and systematic arrangement of kidney tissues with well-defined glomerulus were observed. While, in infected Mrigal, the structural integrity of the kidney tissues was lost and well-defined histopathological changes observed on8 dpi, which indicated disease progression with extensive changes in the tissues of this vital organ. The inflammation of nephritic tubules of challenged tilapia exemplified nephritis. The Glomerulopathy and dilation of Bowman's space are the indications of a defective glomerular filtration of blood, which, in turn, hamper the removal of excess wastes and fluids from the kidney. This clearly justified the ability of *A. hydrophila* in causing systemic infection as it contains many putative virulence genes, including those encoding a type 2 secretion system, an RTX toxin, and polar flagella (Sudheesh *et al.*, 2012). The findings of this study are reasonably similar to those observed by Julinta *et al*, (2017) in *C. cirrhosus* intramuscularly challenged with *A. hydrophila*. On the other hand, in *A. hydrophila* challenged *O. mossambicus*, Azad *et al.* (2001) noted aggregation of melanomacrophages centers (MMC) in the pronephros, necrosis of the cells in the renal interstitium, tubular necrosis and glomerular degeneration, edematous degeneration of the tubules, depletion of cells in the tubular interstitium occlusion of the ophisthonephric collecting duct with MMC.

The results of the present study shown cumulative mortality in fingerlings was 80% in non-treated fish at 6 days of post-infection with the bacteria; in this case of fishes the mortality was 30% in treated fish. The RPS value was 63%. The result indicates that treated plant extract can be used through the intramuscular injection for Mrigal fingerlings to protect against bacterial infection. In the liver of infected Mrigal fish, dispersed and necrotized tissue, infiltration of haemocytes, loss of normal architecture of the hepatic tissue, fatty changes in the hepatic parenchyma, inflammation of pancreas as well as pancreatic acinar cells, and disintegration of intrahepatic exocrine pancreatic tissues were commonly observed, which corroborate the observations of several earlier studies conducted on different fish species due to *Aeromonas* infection (Azad *et al.*, 2001; Ghosh and Homechaudhuri, 2012; Al-Yahya *et al*., 2018). Azad *et al.* (2001) documented vacuolation, congestion of hepatic sinuses with blood cells and internal haemorrhages, pyknotic necrosis of hepatocytes in the liver of *A. hydrophila* challenged *O. mossambicus*; while in the pancreas, they observed acinar cell degradation in 3-5 dpi and mild necrosis in the pancreatic acini. On the other hand, Al-Yahya *et al.* (2018) noted massive haemocyte aggregation, pyknotic nuclei in the hepatopancreas and perivascular cuffing of hepatopancreatic haemolymph vessels in *A. hydrophila* infected blue tilapia, *O. aureus*. These changes may lead to a disorder of lipid metabolism in the liver tissues, i.e., lipidosis, possibly associated with toxins and extracellular products such as hemolysin, protease, elastase produced by aeromonads (Yardimci and Aydin, 2011). In contrast, the study by Islam *et al.* (2008) revealed the development of internal tissue abscess characterized by focal necrosis and haemorrhage. According to them, the distribution of bacterial cells all over the hepatic tissue caused massive diffused necrosis represented by vacuolation and atrophy in the liver of fish challenged with *Aeromonas*. The infiltration of haemocytes in the hepatic tissue is a measure of cellular response, which indicated the ability of Mrigal to respond to the *A. hydrophila* infection.

A. hydrophila can cause serious pathology in the kidney, liver, gill and spleen of *C. cirrhosus* similar to those of other known fish bacterial pathogens such as *A. caviae* (Azad *et al.*, 2001; Julinta *et al.*, 2017; Al-Yahya *et al.*, 2018) or *S. agalactiae* (Adikesavalu *et al.*, 2017). The challenged Mrigal showed haemorrhages at the site of infection and experienced 80% mortalities.

The main visceral organs, viz., kidney, liver, gill and spleen were affected in the infected fish, thus revealing the fact that *A. hydrophila* was able to infect many visceral organs similar to *Aeromonas caviae* like bacterium (Thomas *et al*., 2012) .Systemic infections are generally characterized by diffused necrosis in several internal organs, primarily the liver and kidney are the target organs of an acute septicemia. These organs when attacked by bacterial toxins cause acute haemorrhage and necrosis of vital organs and lose their structural integrity (Huizinga *et al*., 1979). Anwesha Roy *et al*., 2018 described in the control and plant treated fish, the normal structure and systematic arrangement of kidney tissues with well-defined glomerulus were observed. While, in infected tilapia, the structural integrity of the kidney tissues were lost and well-defined histopathological changes observed on 6 dpi, which indicated disease progression with extensive changes in the tissues of this vital organ. The inflammation of nephritic tubules of challenged Mrigal exemplified nephritis. The Glomerulopathy and dilation of Bowman's space are the indications of a defective glomerular filtration of blood, which, in turn, hamper the removal of excess wastes and fluids from the kidney. This clearly justified the ability of *A. hydrophila* in causing systemic infection as it contains many putative virulence genes, including those encoding a type 2 secretion system, an RTX toxin, and polar flagella (Sudheesh *et al.*, 2012). The findings of this study are reasonably similar to those observed by Julinta *et al.*, (2017) in *C. cirrhosus* intramuscularly challenged with *A. hydrophila*, Azad *et al*., (2001) noted aggregation of melanomacrophages centers (MMC) in the pronephros, necrosis of the cells in the renal interstitium, tubular necrosis and glomerular degeneration, edematous degeneration of the tubules, depletion of cells in the tubular interstitium occlusion of the ophisthonephric collecting duct with MMC.

The results of the present study the Kidney of control and treated Mrigal showed normal organization and kidney tubules, hematopoietic tissue and glomeruli were arranged in normal fashion. The kidney of the experimentally infected Mrigal with *A. hydrophila* bacteria developed hematopoietic atrophy with vacuolations and glomerular degeneration. The Gills of control showed normal organization with lamellar epithelium, infected gill showed branchitis at lamellae and hyperplasia, treated gill showed normal gill lamellae. The gills revealed mild proliferation of the covering epithelium particularly at the base of the secondary lamellae and haemocytic infiltration and congestion The Liver of control Mrigal contained neither any vacuoles nor any necrosis, atrophy, hypertrophy or phyknotic cell rather all the hepatocytes were arranged in normal texture. Hepatocytes of the experimentally infected Mrigal fish were evidenced by uncoordination of the tissue. Bacterial interference in the hepatocytes caused vacuolation and hemorrhage, treated Mrigal showed normal structure.

Conclusion

Mrigal (*Cirrhinus cirrhosus*), one of the major Indian carp been frequently infected by a serious bacterium known *Aeromonas hydrophila*. Due to its high pathogenecity, it is taken in to consideration for the effective control. The control includes various elements such as antibiotics, probiotics and medicinal plant extracts. It is economically important to control and prevent the infection for further culture techniques. *Aeromonas hydrophila* is the virulent motile *aeromonad*, which may affect various physiological functioning of Mrigal. Usually, *A. hydrophila* occurs in poor water quality, stressors, temperature fluctuations etc. The invasion of bacteria is indicated by number of morphological and genetic changes in the fish. It is important to identify the risk factors responsible for *Aeromonas* outbreak in Mrigal fish. The antibacterial activity of *Lantana camara* is almost effective for the control of the infection in Mrigal. The ethanolic extract consists of various phytochemical components that help in the treatment of infection. We would recommend the plant extract to treat the infection in Mrigal fingerlings.

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