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# Phytotherapy of *Aeromonas hydrophila*-infected Goldfish, *Carassius auratus*

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## Abstract

**Etiological investigations have often implicated *Aeromonas hydrophila*, a heterotrophic Gram-negative pathogenic aquatic bacterium, in a variety of systemic and localized fish diseases. The aim of this study was to test the efficacy of a herbal concoction against *A. hydrophila*-infected goldfish (*Carassius auratus*). After the pathogen was intramuscularly injected, scales sloughed off on the site of administration, with the appearance of a muscular hemorrhagic protuberance that progressed into an extensive ulcerative dermatitis associated with focal hemorrhage, edema, and dermal necrosis exposing the underlying muscles. Progression of the disease affected the organs in the following order: muscle, gills, liver, and finally the heart tissue. The dip treatment with the concoction (1% herbal concoction dip treatment for 5 min/d daily at 1100 h) restored the histoarchitecture of the altered primary gill lamellae, liver, heart, and muscle. The recovery changes are reported.**

In warm-water fishes, red-sore disease is caused by the Gram-negative bacterium *Aeromonas hydrophila* (Huizinga et al. 1979). The pathogenesis and histopathology of red-sore disease have been extensively studied in the common carp, *Cyprinus carpio* (Amalacher 1970; Wolke 1975), and the Channel catfish, *Ictalurus punctatus* (Gaines 1972). *A. hydrophila* has been linked to red-fin disease in cultured eel, *Anguilla japonica* (Hoshina 1962); red disease in common carp (Egusa 1978); red-sore disease in largemouth bass, *Micropterus salmonides* (Huizinga et al. 1979); and hemorrhagic septicemia in several fish species (Miyazaki and Kaige 1985). In Japan, *A. hydrophila* infection in cultured ayu, *Plecoglossus altivelis*, was linked to exophthalmia and cutaneous hemorrhage in the tail and anus (Jo and Oonishi 1980; Miyazaki and Jo 1985). Although the etiology of epizootic ulcerative syndrome (EUS), another globally devastating fish disease, is uncertain, it is believed that *A. hydrophila* contributes to the pathogenesis of the lesions (Costa and Wijeyaratne 1989). Histopathologic changes in diseased animals provide a methodological platform to determine the causes of mortality (Jagadeesan et al. 1994). Histopathologic changes in *A. hydrophila*-infected catfish, *Clarias batrachus*, and *Salmo gairdneri* have been documented earlier (Angka 1990; Candan 1990). The liver is the major role of metabolism that is involved in breaking down toxic materials and leads hepatic cells to be subjected to more damage than other organs (Pazhanisamy 2002). In largemouth bass, *Micropterus salmoides*, *A. hydrophila* infection leads to necrosis of the liver, kidney, and heart (Huizinga et al. 1979).

In the search for alternative, nonresistant, and cost-effective fish-disease therapeutic agents, Indian medicinal plants are receiving increasing

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attention (Harikrishnan et al. 2003). Harikrishnan and Balasundaram (2005) examined the antibacterial capability of an aqueous herbal concoction in arresting the *in vitro* growth of *A. hydrophila*. Extract obtained from the rhizomes of *Utleria salicifolia* has been shown to improve the healing of ulcer in different animals (Rao et al. 2004), and *Dombeya buettneri* aqueous leaf extract has been shown to be beneficial in healing peptic ulcers (Okwari et al. 2000). The objective of this study was to treat *A. hydrophila*-infected goldfish (*Carassius auratus*) with aqueous herbal concoction obtained from three medicinal plants (Neem, Tulsi, and Turmeric), and to examine histopathologic and recovery changes in lateral muscle, gills, liver, and heart tissues.

## Material and Methods

### *Fish*

Goldfish, *C. auratus* (weight $15 \pm 2$ g/fish; $n = 250$), were collected from a local ornamental fish farm in Tiruchirapalli, India. The fish were acclimatized in 200 L tanks/50 fish (salinity $0.25 \pm 0.5$ ppt, pH $8 \pm 0.5$, temperature $24 \pm 2$ C; dissolved oxygen $6.1 \pm 0.4$ mg/L; photoperiod: light : dark cycle of 1400:1000 h) provided with continuous aeration. During the holding period (15 d), the fish were fed with pelletized diet (25% crude protein) at 5% of their body weight once a day at 1000 h, two thirds of water was replaced daily after siphoning out the unfed and fecal materials. After the holding period, the fish were dipped in 25 ppm formalin for 15 sec with no mortalities and released into freshly prepared experimental tanks. They were further acclimated to these conditions for at least 15 d prior to the commencement of experiments.

### *Pathogen*

*Aeromonas hydrophila* (MTCC 646) was obtained from the Institute of Microbial Technology, Chandigarh, India, and maintained in the laboratory under standard conditions (Harikrishnan et al. 2003). Subcultures were preserved on tryptic soy agar (TSA: w/v; Himedia, Mumbai, India) in slopes at 5 C and routinely tested for pathogenesis (Joseph and Carnahan 1994) by inoculation (Davis and Hayasaka 1983) into goldfish. The stock culture was stored at −70 C in 0.85% NaCl with 20% glycerol (v/v) in tryptic soy broth (TSB: w/v; Himedia) to provide a stable inoculate throughout the experimental study (Chabot and Thune 1991; Yadav et al. 1992). Subcultured *A. hydrophila* was taken from TSA slope and harvested in TSB. The broth was incubated for 24 h in a shaker at 25 C and then centrifuged at 10,000 *g* for 20 min at 4 C (Harikrishnan et al. 2003). The supernatant was discarded and the bacterial pellet was washed three times with phosphate-buffered saline (PBS) at pH 7.2 (Yadav et al. 1992).

### *Preparation of Herbal Concoction*

Fresh leaves of *Azadirachta indica* (Neem), *Ocimum sanctum* (Tulsi), and *Curcuma longa* (Turmeric) were collected in August 2003 from the Bharathidasan University campus, Tiruchirapalli, India. They were surface sterilized separately with 0.1% mercuric chloride (w/v) solution for 10 min and washed thoroughly in running tap water for 10 min, followed by shade drying for about 10 d till weight constancy was achieved (Harikrishnan et al. 2003). Each sample was finely powdered in an electric blender. Ten grams each of *C. longa*, *O. sanctum*, and *A. indica* powder was evenly mixed manually in the ratio of 1:1:1. From this mixture, a sample of 10 g was weighed, soaked in 100 mL sterile water and left under standard conditions for a period of 1 wk (Iwalokun et al. 2001; Harikrishnan et al. 2003). The mixture was then serially filtered through Whatman No. 1 and finally through 0.2 µm filters (Colorni et al. 1998), and the obtained concoction was stored in a sterile bottle (Ilori et al. 1996) until use.

### *Experimental Design*

Goldfish, *C. auratus* ($n = 250$), were initially divided into control ($n = 50$) and experimental (two groups of $n = 50$ each) groups. The control fish were intramuscularly injected with 50 µL of PBS, and the experimental fish were injected with 50 µL of PBS, followed by

50 µL of *A. hydrophila* ($10^3$ colony-forming units/mL). After 3 d, the same dose was repeated. On Day 12 postinfection, the fish were further divided into two groups: Group I, infected untreated ($n = 50$), and Group II, infected and subjected to 1% herbal concoction dip treatment ($n = 50$) for 5 min/d at 1100 am. Three fish were randomly chosen from each group on Days 18, 24, 30, and 36, and euthanized with MS-222 (Sigma, St. Louis, MO, USA) at 0.125 mg/L during injection to collect tissue samples of muscle, gill, liver, and heart for histopathologic analysis.

### *Histopathology*

Tissues of lateral muscle (1–1.5 $cm^2$) from the site of injection, gills, liver, and heart were removed aseptically, processed through graded alcohol, and embedded in paraffin wax. Sections (5–6 µm) were then cut, dried, dewaxed, dehydrated, and stained with hematoxylene and eosin (H&E). The stained sections were dehydrated, cleared in xylene, mounted using DPX resin, and observed under a light microscope (Olympus Optical Co., Ltd, Tokyo, Japan).

## Results

### *Muscle*

Histologic structure of lateral musculature of control *C. auratus* comprises two compactly arranged distinct layers, with an outer superficial red muscle layer and an inner white muscle layer (Fig. 1A). There was a gradual infiltration of macrophage (IMP) on Day 18 postinfection (Fig. 2A). In the infected untreated group (Group I), the underlying lateral musculature showed dermal lesions and subdermal necrosis that extended deeper into the underlying skeletal muscle, accompanied with moderate edema and muscular necrosis (MUN) on Day 24 postinfection (Fig. 2B). On Day 30 postinfection, fragmentation of muscle fibers was noticed along with the occurrence of sarcoplasmic debris, granulomatous inflammation (GI) in the epidermis, epidermal necrosis, and hyperplasia (Fig. 2C). After a period of 36 days of infection, lateral musculature became markedly edematous (E), characterized by cloudy or whitened muscle fibers (Fig. 2D). In the infected-treated group (Group II) on Day 18 (6th day of treatment), regenerative responses began with the appearance

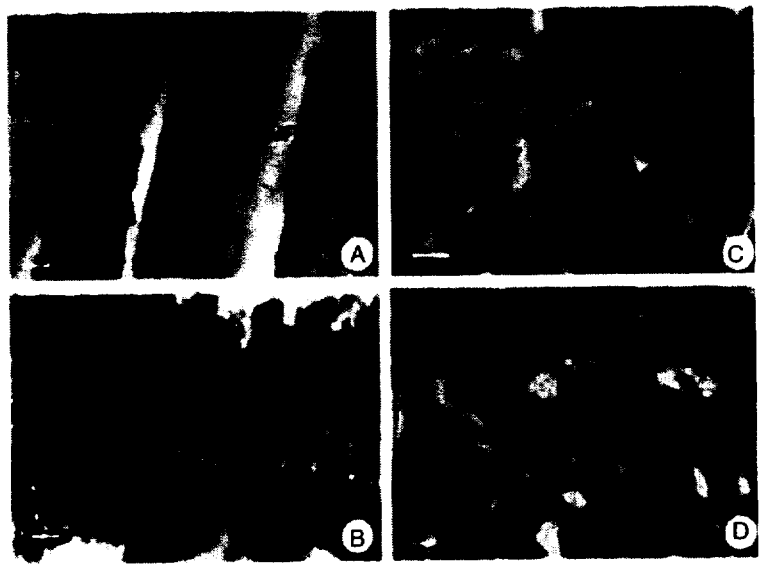


FIGURE 1. *Sectional view of control (normal) muscle, gill, liver, and heart of* Carassius auratus. *(A) Lateral skin musculature of control fish normal muscle fibers (MF) (scalebar = 40 µm). (B) Histologic structure of gill of control fish primary lamellae is lined on either side by the multilayered respiratory epithelium (RE) and leaf-like structure of secondary lamellae (scale bar = 50 µm). (C) Histology of liver of uninfected control fish. Hepatocytes (HC) are large and polygonal in shape (scale bar = 50 µm). (D) Normal histology of heart of uninfected control fish (scale bar = 50 µm).*

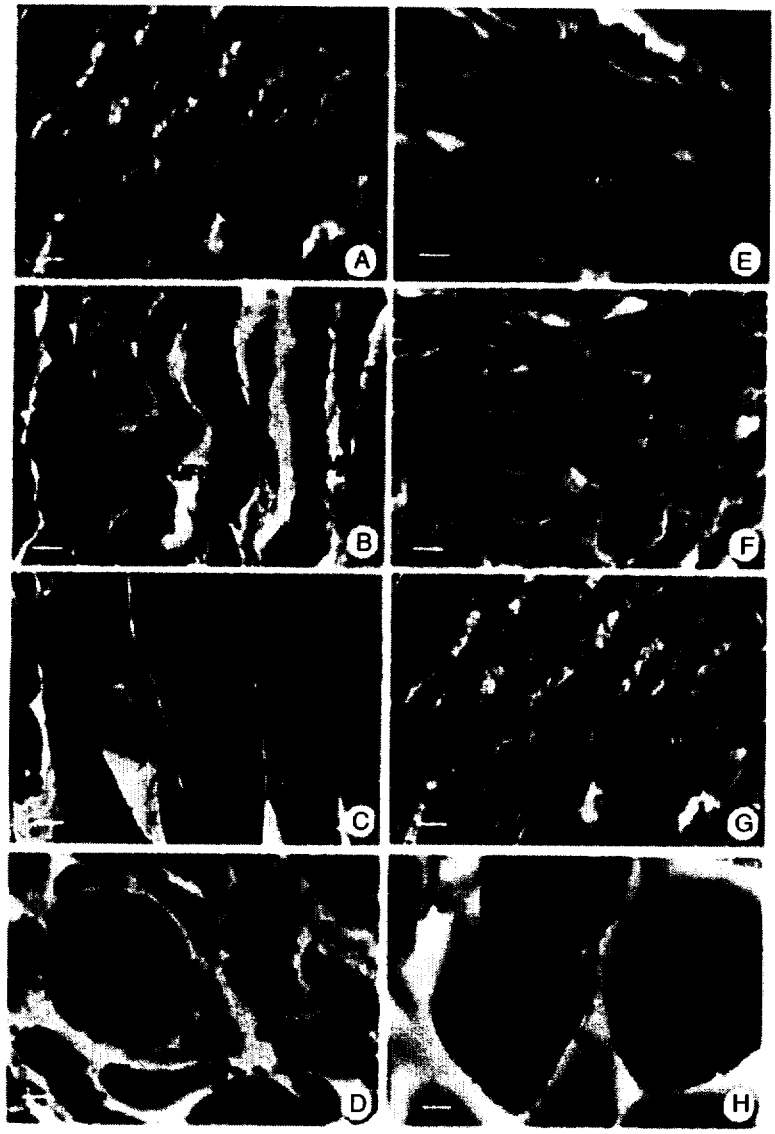


FIGURE 2. *Sectional view of lateral musculature of* Aeromonas hydrophila-*infected untreated and herbal-treated goldfish,* Carassius auratus *(stained with hematoxylin and eosin). (A) Lateral musculature appearance of fibrotic cells (FC) on the 18th day (scale bar = 50 μm). (B) Muscular necrosis (MUN) on the 24th day in infected fish (scale bar = 50 μm). (C) Fragmentation of muscle fibers and sarcoplasmic debris on the 30th day in infected fish (scale bar = 50 μm). (D) Edema (E) and segmentation of muscle fibers on the 36th day in infected fish (scale bar = 50 μm). (E) Regenerative response in treated fish on the 18th day – appearance of macrophages (MP) (scale bar = 50 μm). (F) Regenerative response in treated fish on the 24th day – proliferation of interstitial cells (ISCs) (scale bar = 50 μm). (g) Regenerative response in treated fish on the 30th day – appearance of MP and fibrotic cells (FC) (scale bar = 50 μm). (H) Regenerative response in treated fish on the 36th day – appearance of FC (scale bar = 50 μm).*

of macrophages (MP) (Fig. 2E), which was followed by the proliferation of interstitial cells (ISCs) on Day 24 (Fig. 2F). Further regenerative responses were observed on Days 30 and 36, with the appearance of macrophages and fibrotic cells (Fig. 2G, H).

### *Gills*

Histologic structure of gills in control goldfish was characterized by the presence of primary and secondary lamellae, with mucus cells lying scattered on both sides. The primary lamellae were thickened; the secondary lamellae were

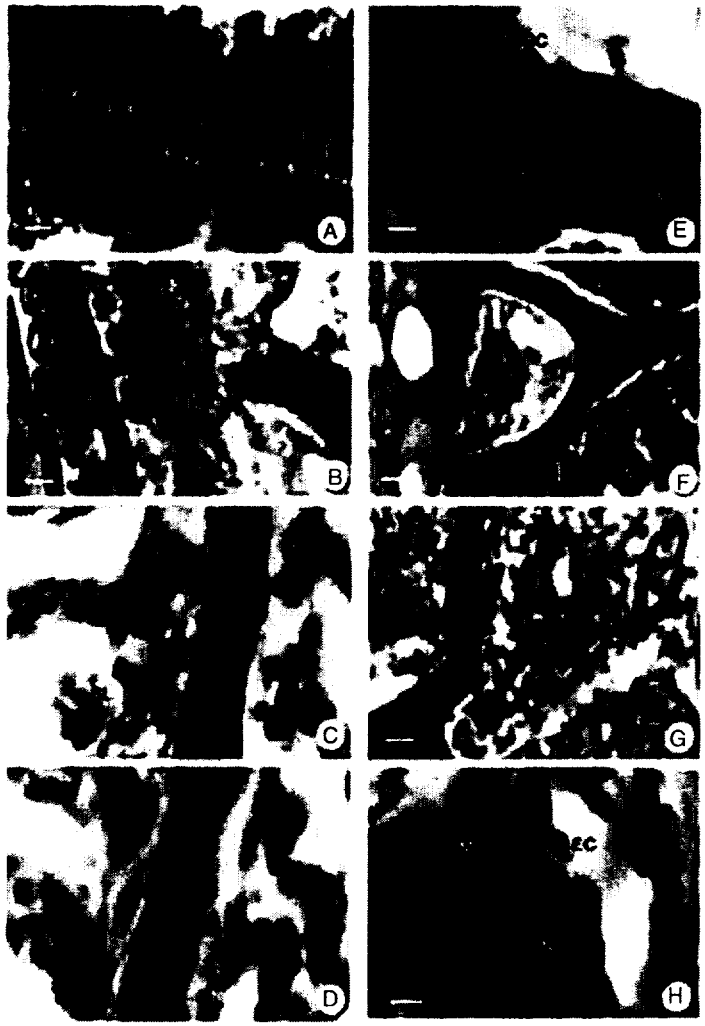


FIGURE 3. *Sectional view of gills of* Aeromonas hydrophila*-infected untreated and herbal-treated goldfish,* Carassius auratus *(stained with hematoxylin and eosin). (A) Proliferation of granulocytes (neutrophils and monocytes) in gill on the 18th day (scale bar = 50 μm). (B) Histopathologic alteration of gill showing edematous lamellae (LE) with bacterial invasion into the capillaries, gill congestion (GC), and hemorrhagic hyperemia (HA) on the 24th day in infected fish (scale bar = 40 μm). (C) Sloughing (SL) of the respiratory epithelium (RE) and formation of hemorrhage globe (HG) on the filament on the 30th day in infected fish (scale bar = 50 μm). (D) Formation of hemorrhagic necrosis (HN) and necrosis (N) seen on the 36th day in infected fish (scale bar = 50 μm). (E) Early regenerative response in treated fish on the 18th day – proliferation of granular cells and thickening of the secondary lamellae (scale bar = 50 μm). (F) Regenerative response in treated fish on the 24th day – moderate hypotrophy (MHT) of lamellar epithelium and necrosis (N) (scale bar = 50 μm). (G) Regenerative response in treated fish on the 30th day – fibrosis and infiltration of the leucocytes (neutrophils and monocytes) (scale bar = 50 μm). (H) Regenerative response in treated fish on the 36th day – HN (scale bar = 50 μm).*

uniform in length, straight, and evenly distributed. The primary and secondary lamellae were also covered by a single thin layer of epithelium (Fig. 1B). Hemorrhagic necrosis (HN) appeared by Day 18 postinfection (Fig. 3A) and on Day 24 postinfection, histologic analysis revealed irregular aggregates of macrophages in gill lamellae, bacterial invasion into the capillaries of the edematous lamellae (LE), gill congestion (GC), and hemorrhagic hyperemia (HA) (Fig. 3B). The lesions were found to be associated with thrombosis, edema, hemorrhage, sloughing (SL) off of the respiratory epithelium (RE), and formation of

hemorrhage globe (HG) on Day 30 postinfection (Fig. 3C). The disease had progressed to degeneration with necrosis of lamellar epithelial cells, diffuse-mixed infiltration of neutrophils with macrophages, and formation of HN and necrosis (N) on Day 36 postinfection (Fig. 3D). In the infected-treated group, histologic analysis revealed proliferation of granular cells, thickening of the secondary lamellae, and infiltration by leucocytes on Day 18 of treatment (Fig. 3E). Moderate hypertrophy (MHT) of lamellar epithelium and minimal necroses (N) were visible on Day 24 of treatment (Fig. 3F). By Day 30 of treatment, infected gills exhibited fibrosis or fibrogranulation tissue, and infiltration of leucocytes such as neutrophils and monocytes (Fig. 3G). In contrast, by Day 36 of treatment aneurism was noted (Fig. 3H).

*Liver*

The liver of the control goldfish showed that hepatic cells were large and polygonal in shape with almost centrally placed nuclei blood sinusoids (SS) (Fig. 1C). By Day 18 postinfection, the specimens exhibited multiple fibroma and macrophage granuloma (MG) (Fig. 4A) and on Day 24 postinfection, the liver of the infected fish showed deep vacuolization and granulation in the cytoplasm, pyknosis of the hepatocyte nuclei, necrosis, GI, and a large number of macrophages and fibroblast (Fig. 4B). The liver exhibited focal necrosis of the hepatocytes with tubular degeneration of the intestinal microvilli and hepato-cellular necrosis (HCN) on Day 30 postinfection (Fig. 4C). By Day 36 postinfection, the liver tissue appeared edematous and congested, with necrotic foci showing fibrin deposition or slight hemorrhage in the pulp, inflammation, free pregranulomatous tissue, and mature granuloma (MAG) (Fig. 4D). Fish exhibited initial regenerative response by Day 18 of treatment (Fig. 4E) and on Day 24, dilation of SS had ceased and formation of normal liver parenchyma gained momentum, indicating a regenerative response (Fig. 4F). Large-scale granulocytes (GC) were noticeable on Day 24 of treatment (Fig. 4H), and by Day 36, formation of normal liver parenchyma was in the advanced stage (Fig. 4G).

*Heart*

The heart in the control fish did not show any pathologic changes (Fig. 1D). Prominent pathologic changes were observed in the heart tissue of the infected fish. Marked hemorrhage and massive necrosis (MN) were evident on Day 18 postinfection (Fig. 5A) and on Day 24 postinfection, the specimens exhibited early signs of heart damage with spongiform and compact mycocardium. Sarcoplasm showed separation of myofibrils, mild hypereosinophilia with fragmentation of cardiac muscle fibers, and IMPs and erythrocytes (EC) (Fig. 5B). Extensive heart damage was severe hyperplasia of pericardium and the presence of leukocyte-like cells in the pericardial cavity, macrophages in loose irregular aggregates, massive necrosis, and myocardial lesion (ML) on Day 30 postinfection (Fig. 5C). By Day 36 postinfection, the affected pericardium exhibited slight-to-severe thickening because of hyperplasia and the presence of a variety of pleomorphic cells in the fibrous layer, fragmented myofibrils bundles; many degenerated mitochondria, granular degeneration (GD), and focal hemorrhage (FH) were noticed (Fig. 5D). Although there was no evidence of active bacterial cell multiplication in necrotic lesions from Days 18 to 24 of treatment period, there was initial regenerative response with dense infiltration of granulocytes (Fig. 5E, F). Marked regenerative responses were evident with dense infiltration of granulocytes on Day 30 of treatment (Fig. 5G) and advanced regenerative response on Day 36 (Fig. 5H).

## Discussion

After Day 24 of infection, the goldfish exhibited fragmentation of muscle fibers, with the occurrence of necrosis and sarcoplasmic debris. There were no external signs of infection, such as the appearance of skin ulcers, in the early stages. The histopathologic changes observed in this study were similar to that in the channel catfish *I. punctatus* (Bach et al. 1978) and

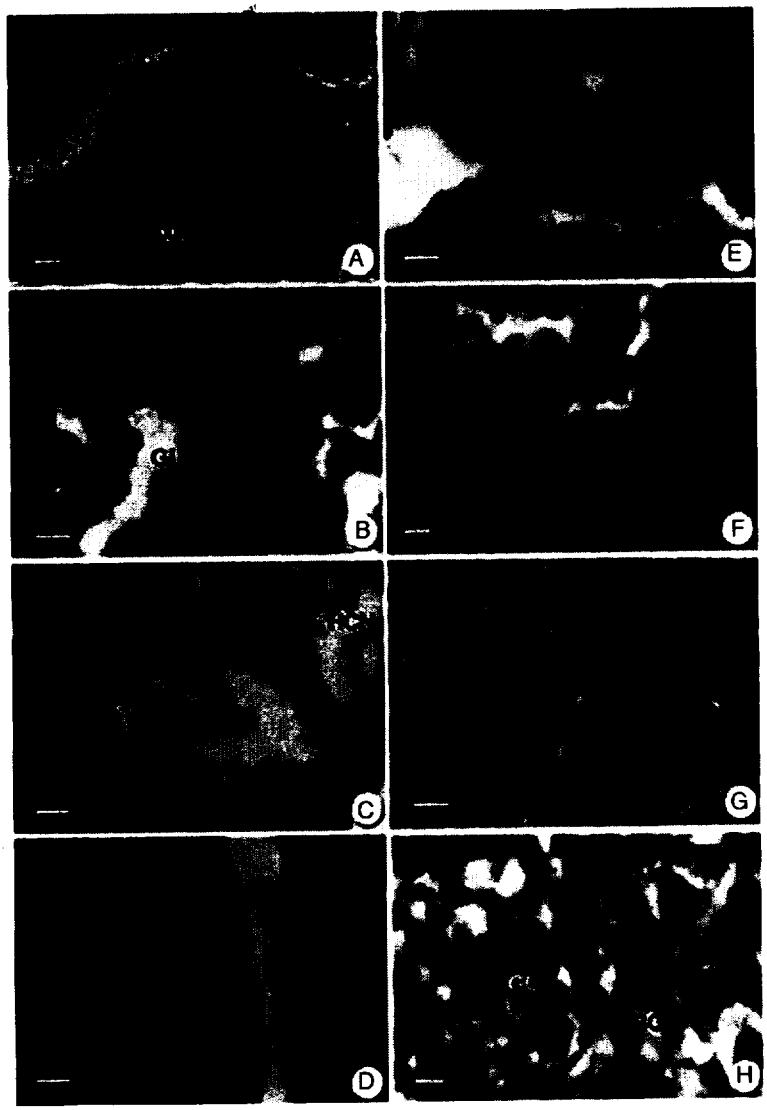


FIGURE 4. *Sectional view of the liver of* Aeromonas hydrophila-*infected untreated and herbal-treated goldfish,* Carassius auratus *(stained with hematoxylin and eosin). (A) Macrophage granuloma (MG) in liver parenchyma on the 18th day (scale bar = 50 μm). (B) Granulomatous inflammation (GI) of liver, large number of macrophages, and fibroblasts on the 24th day in infected fish (scale bar = 50 μm). (C) Hepato-cellular necrosis (HCN) on the 30th day in infected fish (scale bar = 50 μm). (D) Inflammation of liver, free pregranulomatous tissue, and mature granuloma (MAG) on the 36th day in infected fish (scale bar = 50 μm). (E) Initial regenerative response of treated fish on the 18th day (scale bar = 50 μm). (F) Regenerative response in treated fish on the 24th day – diffuse dilation of sinusoids (SS) (scale bar = 50 μm). (G) Regenerative response in treated fish on the 30th day – formation of normal liver parenchyma (scale bar = 50 μm). (H) Late regenerative response in treated fish on the 36th day – large-scale appearance of granulocytes (GC) (scale bar = 50 μm).*

the rainbow trout *S. gairdneri* (Candan 1990) infected with *A. hydrophila*, and *C. carpio* infected with viremia-associated ana-aki-byo (corona-like virus) (Miyazaki et al. 2000). We noticed signs of severe infection after Day 30, with the presence of macrophages in lateral muscle and the proliferation of ISCs. By Day 36 of infection, fibrotic and ISCs were easily identifiable. The largemouth bass *M. salmoides* infected with *A. hydrophila* also showed epidermal necrosis and extensive hyperplasia along with proliferation of fibrocytes and infiltration of inflammatory cells that extended upward from the edematous tissues (Huizinga et al.

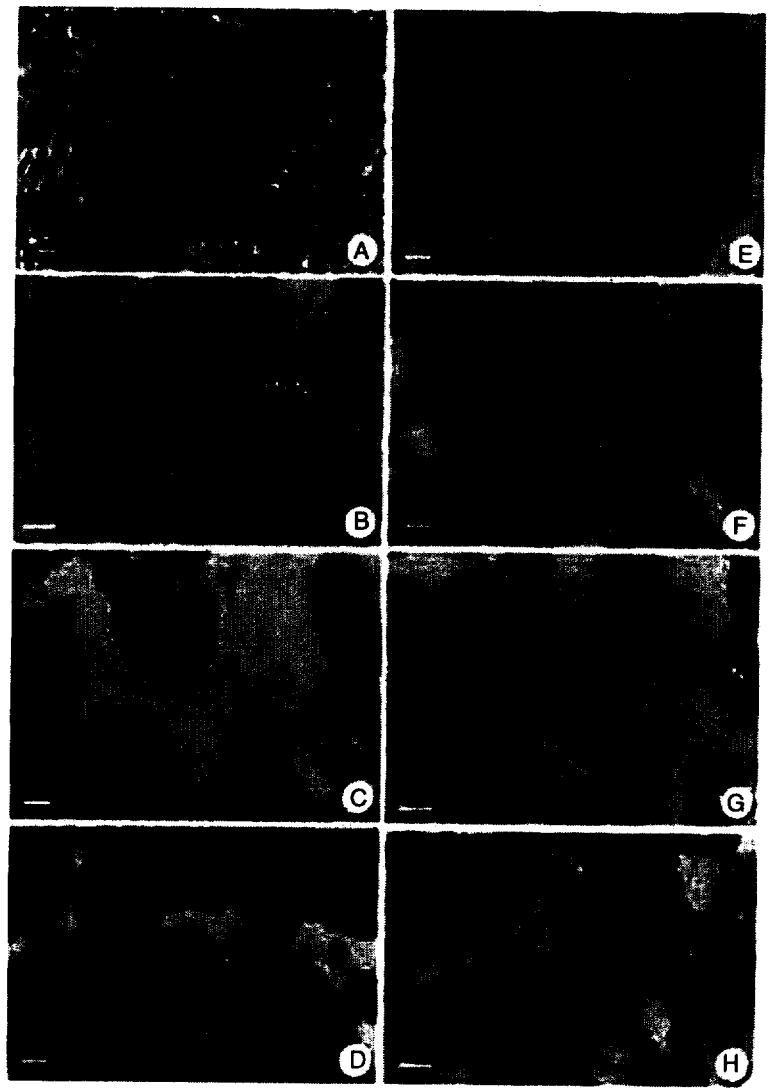


FIGURE 5. *Sectional view of the heart of* Aeromonas hydrophila*-infected untreated and herbal-treated goldfish.* Carassius auratus *(stained with hematoxylin and eosin). (A) Marked hemorrhage and massive necrosis (MN) in heart on the 18th day (scale bar = 50 μm). (B) Fragmentation of cardiac muscle fibers and infiltration of macrophages (IMPs) and erythrocytes (EC) on the 24th day in infected fish (scale bar = 50 μm). (C) Massive necrosis and myocardial lesion (ML) on the 30th day in infected fish (scale bar = 50 μm). (D) Granular degeneration (GD) and focal hemorrhage (FH) on the 36th day in infected fish (scale bar = 50 μm). (E) Initial regenerative response in treated fish on the 18th day – dense infiltration of granulocytes (scale bar = 50 μm). (F) Regenerative response in treated fish on the 24th day – dense infiltration of granulocytes (scale bar = 50 μm). (G) Moderate regenerative response in treated fish on the 30th day (scale bar = 50 μm). (H) Advanced regenerative response in treated fish on the 36th day (scale bar = 50 μm).*

1979). In goldfish, *C. auratus* infected with *A. hydrophila* resulted in a higher cumulative mortality of 16.7% by Day 24 of infection, and 33.3% by Day 36; there was no mortality in the control group. However, in infected fish subjected to herbal treatment, mortality was only 3.3% by Day 18 of infection, and 6.7% by Day 36. Similarly, the size of induced ulcer in the infected fish progressively increased, whereas in the treated group the size of the lesion was 0.3 mm on Day 30 postinfection, which completely healed (0.0 mm) by Day 36 (Harikrishnan et al., communicated). This study indicates that the gill lamellae became edematous because of bacterial invasion into the capillaries by Day 24 of infection.

Other marked histopathologic changes observed in the gills of *C. carpio* and *I. punctatus* infected with *A. hydrophila* are hemorrhagic hyperemia, SL off of the RE, and formation of HG (Grizzle and Kirya 1993; Miyazaki et al. 2001).

In this study, histologic analysis of gills after Day 30 of herbal treatment showed the presence of edematous spaces, proliferation of granular cells, thickening of the secondary lamellae, fibrosis, and infiltration of leucocytes. Edematous spaces are important in the activation of mucosal immune response in different fishes (Demers and Bayne 1994; Moore et al. 1998). In contrast, histopathologies of *A. hydrophila*-infected liver were characterized by the development of GI and HCN (a similar type of tissue destruction and the affinity of this bacterium in the liver were reported; see also Huizinga et al. 1979; Grizzle and Kirya 1993; Miyazaki et al. 2001). Ventura and Grizzle (1988), Angka (1990), and Candan (1990) also report that *Clarias bactrachus*, *S. gairdneri*, and *I. punctatus* experimentally infected with *A. hydrophila* were affected with necrosis and hemorrhage in the kidney, liver, pancreas, and intestine. A Tiger Oscar, *Astronotus ocellatus*, infected with motile aeromonad septicemia contained a large amount of red-ascitic fluid accumulated in the abdominal cavity, along with hemorrhages in the liver and kidney (Soltani et al. 1998). In this study, treated fish showed faster regenerative responses such as diffusion of SS dilation and reappearance of normal liver parenchyma by Day 24, and multiple fibroma by Day 36.

Infected fish exhibited fragmentation of cardiac muscle fibers, IMPs, EC, massive necrosis, and ML by Day 24 of infection. In contrast, herbal treated fish showed regenerative responses with dense infiltration of granulocytes in muscle between Days 18 and 36. Recent *in vitro* and *in vivo* experiments in rats suggest that topical antimicrobials may not only be toxic to fibroblasts and keratinocytes, but also retard wound healing (John et al. 1996). In this regard, disease management in fish using phytotherapy is becoming an attractive alternative, given that the raw materials are biodegradable and without side effects (Harikrishnan et al. 2003).

Extensive studies have documented the beneficial effects of herbal treatment in human diseases (e.g., Weiss 1988; Davis et al. 1992; Echinacea 1994; Guillaume and Padioleau 1994; Leung and Foster 1996; Blumenthal et al. 1998; Shukla et al. 1999; Oliveira et al. 2004). Wu et al. (2001) found that weight gain and resistance to common infectious disease of eels, *Anguilla anguilla*, that were fed traditional Chinese medicines (TCMs) increased significantly. Jian and Wu (2004) observed that TCM had a beneficial effect on the growth and for the prevention and treatment for common disease in *C. carpio*. Neem leaves, garlic, and turmeric powder have been used to produce a disease-resistant fry of Indian major carp, *Catla catla* (Dey and Chandra 1995). Herbs have also been tried in other countries for control of shrimp and fish disease, and successful results have been reported in Mexico, Thailand, Japan, and Turkey (Auro de Ocampo and Jimenez 1993; Dey and Chandra 1995; Direkbusarakom et al. 1996; Dugenci et al. 2003). These studies highlight the immense potential of herbal remedies and the need to extend the application of phytotherapy to fish-disease management. In this study, we have shown that herbal application may also be of practical use in disease management strategy in fish. To the best of our knowledge, this is the first detailed investigation to report on the restorative changes in the histoarchitecture of gill, liver, heart, and muscle of goldfish *C. auratus* artificially infected with *A. hydrophila*.

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## Literature Cited

**Amalacher, E.** 1970. Textbook of fish diseases. TFH Publications, Inc., Neptune City, New Jersey, USA.

**Angka, S. L.** 1990. The pathology of the walking catfish, *Clarias batrachus* (L.), infected intraperitoneally with *Aeromonas hydrophila*. Asian Fisheries Science 3:343–351.

**Auro de Ocampo, A. and E. M. Jimenez** 1993. Herbal medicines in the treatment of fish diseases in Mexico. Veterinaria Mexico 24:291–295.

**Bach, R., P. K. Chen, and G. B. Chapman.** 1978. Changes in the spleen of the channel catfish *Ictalurus punctatus* Rafinesque induced by infection with *Aeromonas hydrophila*. Journal of Fish Diseases 1:205–217.

**Blumenthal, M., W. R. Busse, and A. Goldberg.** 1998. The complete German Commission E Monographs. Therapeutic guide to herbal medicines. American Botanical Council, Austin, Texas, USA.

**Candan, A. A.** 1990. A study on the histopathology of *Aeromonas hydrophila* infections of rainbow trout (*Salmo gairdneri* R.) kept under experimental conditions and the effect of chloramphenicol. Journal of Aquatic Production 4:5–20.

**Chabot, D. J. and R. L. Thune.** 1991. Proteases of the *Aeromonas hydrophila* complex: identification, characterization and relation to virulence in channel catfish, *Ictalurus punctatus* (Rafinnesque). Journal of Fish Diseases 14:171–183.

**Colorni, A., R. Avtalion, W. Knibb, E. Berger, B. Colorni, and B. Timan.** 1998. Histopathology of sea bass (*Dicentrarchus labrax*) experimentally infected with *Mycobacterium marinum* and treated with streptomycin and garlic (*Alium sativum*) extract. Aquaculture 160:1–17.

**Costa, H. H. and M. J. S. Wijeyaratne.** 1989. Epidemiology of epizootic ulcerative syndrome occurring for the first time among fish in Sri Lanka. Journal of Applied Ichthyology 1:48–52.

**Davis, J. F. and S. S. Hayasaka.** 1983. Pathogenic bacteria associated with cultured American eels *Anguilla rostrata* Le Sueur. Journal of Fish Diseases 23: 557–564.

**Davis, R. H., G. H. Stewart, and P. J. Bregman.** 1992. *Aloe vera* and the inflamed synovial pouch model. Journal American Podiatric Medical Association 82: 140–148.

**Demers, N. E. and C. J. Bayne.** 1994. Plasma proteins of rainbow trout *Onchorhynchus mykiss* immediate response to acute stress. Pages 1–9 *in* J. S. Stolen and T. C. Fletcher, editors. Models for environmental toxicology biomarkers, immunostimulants, volume 1. SOS Publications, Fair Havan, New Jersey, USA.

**Dey, R. K. and S. Chandra.** 1995. Preliminary studies to raise disease resistant seed (fry) of Indian major carp, *Catla catla* (Ham.) through herbal treatment of spawn. Fish Chimes March 1995:23–25.

**Direkbusarakom, S., A. Herunsalee, M. Yoshimizu, and Y. Ezura.** 1996. Antiviral activity of several Thai traditional herb extracts against fish pathogenic viruses. Fish Pathology 31:209–213.

**Dugenci, S. K., N. Arda, and A. Candan.** 2003. Some medicinal plants as immunostimulant for fish. Journal of Ethnopharmacology 88:99–106.

**Echinacea, H. C.** 1994. A literature review. HerbalGram 30:33–48.

**Egusa, S.** 1978. Infectious disease of fish [in Japanese]. Kouseisha Kouseikaku, Tokyo, Japan.

**Gaines, J. L.** 1972. Pathology of experimental infection of *Aeromonas hydrophila* (Chester) Stanier, (Bacterial: *Pseudomonadales*) in the channel catfish, *Ictalurus punctatus* (Rafinesque). PhD Thesis. Auburn University, Auburn, Alabama, USA.

**Grizzle, J. M. and Y. Kirya.** 1993. Histopathology of gill, liver and pancreas and serum enzyme levels of channel catfish infected with *Aeromonas hydrophila* complex. Journal Aquatic Animal Health 5:36–50.

**Guillaume, M. and F. Padioleau.** 1994. Veinotonic effect, vascular protection, anti-inflammatory and free radical scavenging properties of horse chestnut extract. Arzneimittel forschung 44:25–35.

**Harikrishnan, R. and C. Balasundaram.** 2005. Modern trends in *Aeromonas hydrophila* disease management with fish. Review in Fisheries Science 13:281–320.

**Harikrishnan, R., M. Nisha Rani, and C. Balasundaram.** 2003. Hematological and biochemical parameters in common carp, *Cyprinus carpio*, following herbal treatment for *Aeromonas hydrophila* infection. Aquaculture 221:41–50.

**Hoshina, T.** 1962. Studies on Red-Fin Disease of Eel [in Japanese]. Special Research Report of Tokyo University of Fisheries, No. 6. Tokyo, Japan.

**Huizinga, H. W., G. W. Esch, and T. C. Hazen.** 1979. Histopathology of red-sore disease (*Aeromonas hydrophila*) in naturally and experimentally infected largemouth bass *Micropterrus salmoides* (Lacepeda). Journal of Fish Diseases 2:263–277.

**Ilori, M. O., A. O. Sheteolu, E. A. Omonigbehin, and A. A. Adeneye.** 1996. Antidiarrhoeal activities of *Ocimum gratissimum* (Lamiaceae). Journal of Diarrhoeal Disease Research 14:283–285.

**Iwalokun, B. A., G. O. Gbenle, T. A. Adewole, and K. A. Akinsinde.** 2001. Shigellocidal properties of three Nigerian medicinal plants: *Ocimu gratissimum, Terminalia avicennoides*, and *Momordica balsamina*. Journal of Health Population Nature 19: 331–335.

**Jagadeesan, V., N. J. Rao, and B. Sesikeran.** 1994. Effect of iron deficiency on DMH-induced gastrointestinal tract tumors and occurrence of hepatocyte abnormalities in Fischer rats. Nutrition and Cancer 10: 223–285.

**Jian, J. and Z. Wu.** 2004. Influences of traditional Chinese medicine on non-specific immunity of Jian Carp (*Cyprinus carpio* var. *Jian*). Fish and Shellfish Immunology 16:185–191.

**Jo, Y. and K. Oonishi.** 1980. *Aeromonas hydrophila* isolated from ayu. Fish Pathology 15:85–89.

**John, P., H. A. Kucukcelebi, D. Listengarten, J. Stabenau, F. Ko, L. D. Broemeling, M. C. Robson, and W. D. Winters.** 1996. Effect of aloe on wound healing in an excisional wound model. The Journal of Alternative and Complementary Medicine 2:271–277.

**Joseph, S. W. and A. Carnahan.** 1994. The isolation, identification, and systematic of the motile *Aeromonas* species. Annual Review in Fish Disease 4:315–343.

**Leung, A. and S. Foster.** 1996. Encyclopedia of common natural ingredients used in food, drugs and cosmetics. 2nd edition. John Wiley & Sons, New York, New York, USA.

**Miyazaki, T. and Y. Jo.** 1985. A histopathological study of motile aeromonad disease in ayu. Fish Pathology 20:55–59.

**Miyazaki, T. and N. Kaige.** 1985. A histopathological study on motile aeromonad disease in Crucian carp. Fish Pathology 21:181–185.

**Miyazaki, T., H. Okamoto, T. Kageyama, and T. Kobayashi.** 2000. Viremia-associated ana-aki-byo. A new viral disease in color carp *Cyprinus carpio* in Japan. Disease of Aquatic Organisms 39:183–192.

**Miyazaki, T., T. Kageyama, M. Miura, and T. Yoshida.** 2001. Histopathology of viremia-associated ana-aki-byo in combination with *Aeromonas hydrophila* in color carp *Cyprinus carpio* in Japan. Disease of Aquatic Organisms 44:100–120.

**Moore, J. D., M. Ototake, and T. Nakanishi.** 1998. Particulate antigen uptake during immersion immunisation of fish: the effectiveness of prolonged exposure and the roles of skin and gill. Fish and Shellfish Immunology 8:393–407.

**Okwari, O. O., R. R. Ettarh, B. A. Akpogomeh, and M. U. Eteng.** 2000. Gastric anti-secretory and anti-ulcerogenic effects of *Dombeya buettneri* in rats. Journal of Ethnopharmacology 71:315–319.

**Oliveira, F. A., G. M. Vieira-Junior, M. H. Chaves, F. R. Almeida, M. G. Florencio, R. C. Lima, Jr., R. M. Silva, F. A. Santos, and V. S. Rao.** 2004. Gastroprotective and anti-inflammatory effects of resin from *Protium heptaphyllum* in mice and rats. Pharmacological Research 49:105–111.

**Pazhanisamy, K.** 2002. Studies on the impact of Arsenic Trioxide on a freshwater fish, *Labeo rohita* (Hamilton). PhD Thesis. Annamalai University, India.

**Rao, C. V., S. K. Ojha, K. Radhakrishnan, R. Govindarajan, S. Rastogi, S. Mehrotra, and P. Pushpangadan.** 2004. Antiulcer activity of *Utleria salicifolia* rhizome extract. Journal of Ethnopharmacology 91:243–249.

**Shukla, A., A. M. Rasik, and B. N. Dhawan.** 1999. Asiaticoside-induced elevation of antioxidant levels during acute wound healing. Phytotherapy Research 13:50–54.

**Soltani, M., S. S. Mirzargar, and H. A. Abrahizadeh.** 1998. Occurrence of a motile *Aeromonas* septicaemia in the imported ornamental fish, oscar *Astronotus ocellatus*: isolation characterization and pathogenicity. Journal of Faculty of Veterinary Medicine University of Tehran 53:63–65.

**Ventura, M. T. and J. M. Grizzle.** 1988. Lesions associated with natural and experimental infections of *Aeromonas hydrophila* in channel catfish, *Ictalurus puntatus* Rafinesque. Journal of Fish Diseases 11:397–407.

**Weiss, R.** 1988. Herbal medicine. Ab Arcanum and Beaconsfield, Gothenburg, Sweden; Beaconsfield Publishers Ltd, UK.

**Wolke, R. E.** 1975. Pathology of bacterial and fungal diseases affecting fish. Pages 33–116 *in* W. E. Ribelin and G. Migaki, editors. The pathology of fishes. University of Wisconsin Press, Madison, Wisconsin, USA.

**Wu, D. F., S. G. Lin, S. K. Wang, J. S. Li, and Z. J. Huang.** 2001. Effect of feed additives made of Chinese herbal medicine on culture of *Anguilla anguilla*. Journal of Fujian Agricultural University 30: 95–98.

**Yadav, M., G. Indira, and A. Ansary.** 1992. Cytotoxin elaboration by *Aeromonas hydrophila* isolated from fish with epizootic ulcerative syndrome. Journal of Fish Diseases 15:183–189.