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Phytotherapy of experimentally induced gill inflammation with *Aeromonas hydrophila* infection in goldfish, *Carassius auratus*

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Goldfish, *Carassius auratus* (wt 13 g) was intramuscularly infected with *Aeromonas hydrophila* (4.3×10^6 cfu / ㎖). Infected gills showed edematous lamellae with bacterial invasion into the capillaries and gill congestion on 12th day. By 24th day post-infection, histological analysis revealed irregular aggregates of macrophages in gill lamellae, large amount of mucus cells, gill lamellae edematous with bacterial invasion into capillaries, gill congestion and damaged gill epithelium with hyperplasia. Inflammation of the gill filament and hemorrhage globe was associated with the development of severe necrosis on the 36th day in the infected fishes. In infected and herbal treated fish the regenerative responses like fibrosis and infiltration of the leucocytes (neutrophils and monocytes) occurred on 12th day; moderate hypertrophy in the gills was noticed on the 36th day. These results suggest that phytotherapy ensures better protection and regenerative response against *A. hydrophila* infection in goldfish, *C. auratus.*

Key words: Goldfish, Gills, Histopathology, Herbal

Introduction

Aeromonas hydrophila has emerged as one of the most economically important bacterial infection in fresh water fishes (Harikrishnan and Balasundaram, 2005). *A. hydrophila* infected fishes are anorexic and display abnormal swimming behavior, autopsy finding typically include a pale heart, yellow liver, ascites and swollen spleen (Kongtorp *et al*., 2004). Donta and Haddow (1978) have demonstrated that the toxins of *A. hydrophila* are cytotoxic. Histopathological changes of diseased fish are promising tools to determine the causes of stress or disease (Pazhanisamy, 2002). Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants (Thophon *et al*., 2003) and field studies (Schwaiger *et al*., 1997; Teh *et al*., 1997). Histopath ological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs, including gills, kidney and liver, that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish (Gernhofer *et al*., 2001). Furthermore, the alterations found in these organs are normally easier to identify than functional ones (Fanta *et al*., 2003), and serve as warning signs of damage to animal health (Hinton and Lauren, 1990). Gills are very vulnerable to aeromonad infection teleosts which cause injury

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and subsequent dysfunctions of respiration and osmoregulation (Ellis *et al*., 1989) and channel catfish, *Ictalurus punctatus* infected with *A. hydrophila* had diffuse necrosis in several internal organs (Ventura and Grizzle, 1988). Application of antibiotics, chemotherapy and vaccination in fish disease management may partially control (Harikrishnan, 2003).

In recent years, the application of phytotherapy to fish diseases has attracted much attention (Harikrishnan *et al*., 2005). Medicinal plants have been used in traditional systems to treat many diseases (Bhadauria *et al*., 2002) including fish diseases (Harikrishnan *et al*., 2003). Crude aqueous extracts of neem stem bark increases the production of migration inhibition of lymphocytes in man (Van der Nat *et al*., 1987), anti-inflammatory activity (Sadekar *et al*., 1998) and immunostimulant activity (Sen *et al*., 1992) in rats. Neem seed oil has been shown to cures various skin infections (Chopra *et al*., 1956). It contains several active compounds like nimbin, nimbinin, nimbidinin and nimbidic acid (Mitra *et al*., 1971). Similarly, *Ocimum sanctum* has a vast number of therapeutic applications such as cardiopathy, homeopathy, leucoderma, asthma, bronchitis, catarrhal fever, hepatopathy, nausea, lumbago, hiccups, skin diseases, and the aqueous leaf extract as antibacterial activity (Gupta *et al*., 2002). It contains several compounds for example curcumin essential oil from the seeds has anti-inflammatory (Anto *et al*., 1998), antioxidant (Selvam *et al*., 1995), antimicrobial (Janssen *et al*., 1989), antibacterial (Nakamura *et al*., 1999), antiulcer (Singh *et al*., 1999) and help in fighting a number of diseases like cancer and Acquired Immuno Deficiency Syndrome (AIDS) (Gupta *et al*., 2002).

Although this plant is being widely used in human medicine in India but unfortunately only a few reports on its application in preventing fish diseases (Harikrishnan *et al*., 2003, 2005). Hence, probably for the first time, an investigation aimed to study the effect of aqueous tri-herbal phytotherapy on the restorative changes in gills of goldfish (*C. auratus*) infected with *A. hydrophila.*

Materials and Methods

The goldfish, *Carassius auratus* (13 g) obtained from local fish farm in Tiruchirapalli, Tamilnadu, India were used in the present experiments. The fish were transported to the laboratory in plastic bags in separate (20 fish in 5 L) filled with oxygenated water. They were acclimatized in plastic aquaria (25 L capacity) for 3 weeks to laboratory conditions (10 hrs dark: 14 hrs light; temp 28 ± 2℃). The observed water quality parameters are: dissolved oxygen concentration 5.2–7.8 mg / l (Winkler's method), pH. 5.5–7.1. The fish were fed *ad libitum* once daily with formulated standard diet pellet feed at a ration of 2% of their body weight / day throughout the period of study. Partial water exchange was performed daily to remove waste feed and fecal materials. All fish were held in laboratory for at least 15 days prior to use in experiments to allow for acclimatized and evaluation of overall fish health. Only healthy fish, as determined by general appearance and level of activity, were used for the studies (Shao *et al*., 2004).

Aeromonas hydrophila (MTCC 646) was obtained from Institute of Microbial Technology, Chandigarh, India and maintained in the laboratory under standard conditions (Harikrishnan *et al*., 2003). Subcultures were maintained on tryptic soy agar (TSA: w / v; Himedia, Mumbai) slopes at 5℃ and routinely tested for pathogenesis as described by Joseph and Carnahan (1994) by inoculation into goldfish (Davis and Hayasaka, 1983). Stock culture in tryptic soy broth (TSB: w / v; Himedia, Mumbai)

was stored at -70℃ in 0.85 % NaCl with 20% glycerol (v / v) to provide stable inoculate throughout the experimental study (Yadav *et al*., 1992). *A. hydrophila* sub cultured was taken on TSA slope and were harvested in TSB for experiment. The inoculated broth was incubated for 24 h in a shaker at 25℃ and then centrifuged at 10,000 rpm for 20 min at 4℃ (Harikrishnan *et al*., 2003). The supernatant was discarded and the bacterial pellet washed three times with phosphate-buffered saline (PBS) at pH 7.2 (Yadav *et al*., 1992).

Probably 50 goldfish were inoculated intramuscularly with *A. hydrophila* (4.3×10^6 cfu / ㎖) since the infection manifested as lesion at the site of administration (caudal region) on day 6 (Harikrishnan *et al*., 2003). The experimental fish were categories into two groups. Each group containing 25 fish and 5 fish were used for histopathological study. Another 10 fish normal fish (without infection) was used as control group. Group I: Control, Group II: Infected-untreated and Group III: Infected and treated with tri-herbal extract (concoction) ($1㎖\ l^{-1}$) lasted for 5 min daily from the onset of the lesion on day 6. The specific mortalities were confirmed through reisolation of the pathogen from kidney on Rimler Shott's medium containing novobiocin (Shotts and Rimler, 1973).

Fresh leaves of Azadirachta indica, Ocimum sanctum and Curcuma longa collected from the Bharathidasan University campus, Tiruchirappalli, India during May, 2006. About 5 kg were surface sterilized with 0.1 % mercuric chloride (w/v) solution and washed thoroughly in running tap water for 10 minutes (Harikrishnan et al., 2003). The leaves were separately shade dried for 10 day till weight constancy was achieved. Each sample was finely powdered in an electric blender. The aqueous concoctions (mixed herbal extracts) were obtained following the methodology of Iwalokun et al. (2001). The concoction (C. longa + O. sanctum + A. indica) was prepared by evenly mixing the chosen herbal powders in equal quantities of 100 g in even ratio (1 : 1 : 1). From this, 50 gm was soaked in 100 ㎖ sterile water and kept for 7 days at room temperature under sterile conditions. The extract was filtered through Whatman No. 1 filter paper, and then 0.45 μm, and finally 0.2 μm filters (Colorni et al., 1998) and stored in sterile bottles (Ilori et al., 1996) till used.

The gills were removed from fish were anaesthetized with MS-222 (Sigma Chemical Co., St Louis, MO, USA). All experimental groups including controls on 6, 12, 24 and 36 days post challenge (PC) and processed through graded alcohol and embedded in paraffin wax. Sections were cut (5-6 μm) dried, de-waxed, dehydrated and stained with hematoxylene and eosin (H & E). Stained sections were dehydrated, cleared in xylene, mounted using DPX and examined by light microscopy.

Results and Discussion

The fish gill is very sensitive to physical and chemical changes of the aquatic medium. Hocutt and Tilney (1985) have described gill lesions like oedema, epithelia1 desquamation and fusion of lamellae caused by above stress. Pollution or stress has been shown to alter chloride cell structure and induce lamellar epithelium desquamation and / or filament epithelium hyperplasia (Crespo and Sala, 1986). These are close relationship between gill morphological changes and stress (Peters and Hong, 1985) or lack of polyunsaturated fatty acids in the diet (Bell *et al*., 1985) and several infectious agents have been described in association with proliferative gill diseases and gill necrosis (Kovacs-Gayer, 1984). Histological structure of the gills of normal un-infected goldfish was, characterized by

the presence of primary and secondary lamellae with mucus cells lying scattered on both sides. The primary lamellae were thicker than the secondary lamellae. The primary lamellae were lined on either side by a multi-layered inter lamellar epithelium and the leaf-like secondary lamellae were compactly arranged on the cartilaginous gill ray, which runs in the center of the supporting primary lamellae. The tips of the primary gill lamellae were characterized by the presence of broad and flattened epithelial cells on either side of the secondary lamella. Each filament contained a central venous sinus adjacent to the filamental skeleton (Fig. 1).

The gills of infected gold fish exhibited remarkable changes (Fig. 2a-d). One of them had shortening of all filaments with fusion of secondary lamellae at the tip of the filaments with pathological deviations of the gills on 6thday of infection (Fig. 2a). Gill showed edematous lamellae with bacterial invasion into the capillaries and gill congestion (GC) with presence of haemorrhagic hyperemia (HA) on 12th day of infected untreated fishes (Fig. 2b). Observations have suggested that previously affected gill was consistent with that described a similar pathological alteration has been reported in

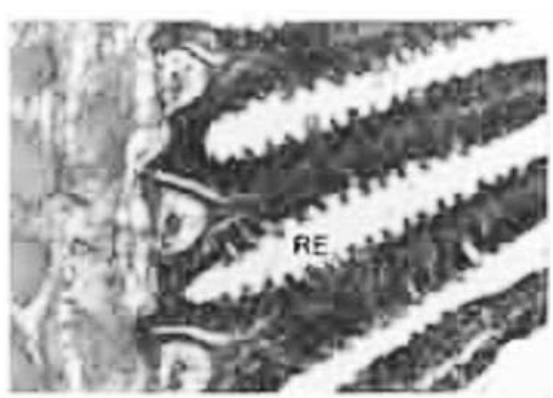

Fig. 1. Histology of *Carassius auratus* control fish gill. Primary lamellae are lined on either side by the multi-layered respiratory epithelium (RE) and with leaf like structure of secondary lamellae (Scale bar=50 m).

ayu (*Plecoglossus altivelis*) vaccinated with *Vibrio anguillarum* the gill showed lamellar edema with bacterial invasion into capillaries, necrotic muscle fibres, hemorrhage infiltration of many inflammatory cells in the subcutaneous adipose tissue and lateral musculature (Miyazaki, 1987). Ventura and Grizzle (1998) also suggest that *Clarias bactrachus*, *Salmo gairdneri* and *Ictalurus punctatus* experimentally infected with *A. hydrophila* were affected with necrosis and hemorrhage in the kidney, liver, pancreas and intestine (Candan, 1990). *Astronotus ocellatus* an ornamental fish infected with motile aeromonad septicamia contained a large amount of red-ascitic fluid accumulated in the abdominal cavity along with hemorrhages in the liver and kidney has previously been reported (Soltani *et al*., 1998). Donta and Haddow (1978) have demonstrated that *A. hydrophila* toxins are cytotoxic and it cause acute hemorrhage and necrosis in the vital organs like liver and kidney, leading to rapid death due to organ failure (Huizinga *et al*., 1979).

Gill morphology is therefore a good indicator of the water quality and the general health condition of cultured fish (Peters *et al*., 1984). Infected fish lamellae large amounts of mucous cells were present in the gill rays, damaged gill epithelium with sloughing (SL) off of the respiratory epithelium and formation of haemorrhage globe (HG) on 24th of infection (Fig. 2c) were found in the present study as suggest that the previous studies, *Oncorhynchus kisutch* affected by coho salmon syndrome (Cold water vibriosis, Hitra disease) the gills showed histopathological alterations like multi-focal epithelial hyperplasia in the secondary lamellae, necrosis within the hyperplastic tissue and occasional occurrence of thrombi and basophilic granules (Branson and Diaz-Munoz, 1991). Similar findings were made in other areas where pathological response reported in color carp, *Cyprinus carpio* artificially

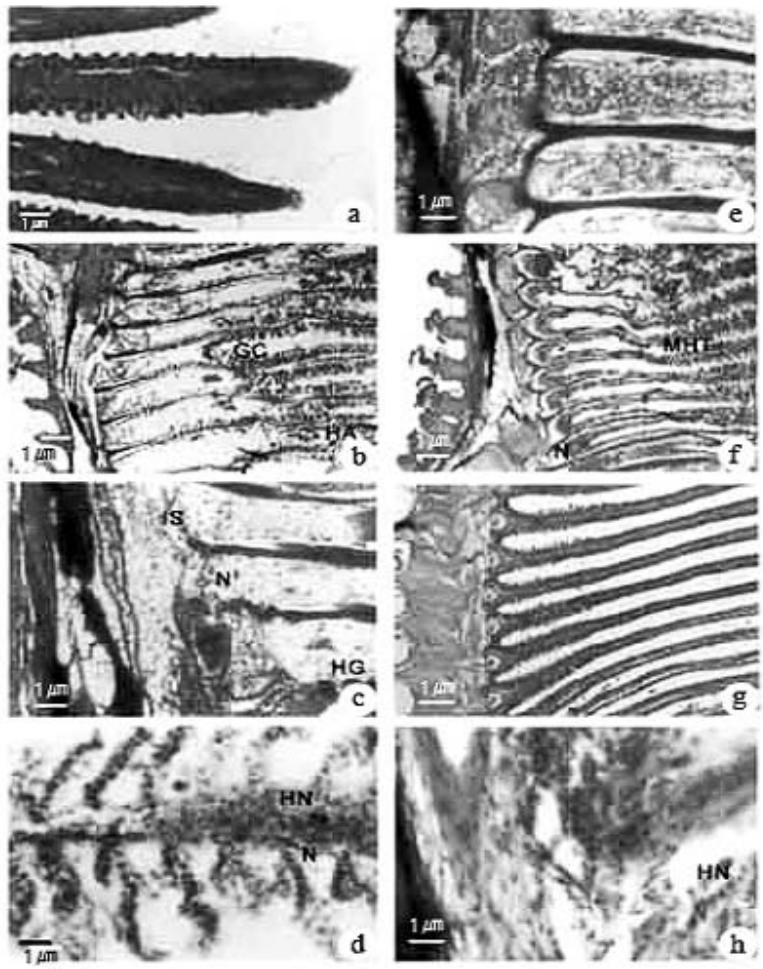

Fig. 2. Changes in *Carassius auratus* gills of *Aeromonas hydrophila* infected and infected with herbal treated. a. Shortening of all filaments with fusion of secondary lamellae on 6th day of infection. b. Showing oedematous lamellae with bacterial invasion into the capillaries, gill congestion (GC) and haemorrhagic hyperaemia (HA) on 12th day of infection (Scale bar=40 m). c. Sloughing (SL) off of the respiratory epithelium and formation of haemorrhage globe (HG) and necrosis (N) on the filament on 24th day of infection (Scale bar=50 m). d. Formation of haemorrhage necrosis (HN) on 36th day of infected (Scale bar=50 m). e. Proliferation of granular cells and thickening of the secondary lamellae on the 6th day of treatment (Scale bar=50 m). f. Moderate hypotrophy (MHT) of lamellar epithelium and necrosis (N) on the 12th day of treatment (Scale bar=50 m). g. Fibrosis and infiltration of the leucocytes (neutrophils and monocytes) on the 24th of treatment (Scale bar=50 m). h. Haemorrhagic necrosis (HN) on the 36th day treatment.

infected with viremia-associated ane-aki-byo in combination with *A. hydrophila* respiratory epithelia and interlamellar epithelia were found separated due to edematous dilation of the choroidal membrane (Miyazaki *et al*., 2001). Furthermore, *Ictalurus punctatus* infected with *A. hydrophila* the gill lymphocytes were found to accumulate in intercellular spaces with slight hyperplasia and spongiosis in lamellar epithelium (Grizzle and Kiryu, 1993).

In *Micropterus salmoides* infected with *A. hydrophila* the gills had severe hemorrhage and necrosis exposing the supporting cartilage, loss of functional integrity of gill tissue, diffuse edema, profuse hemorrhage, mononuclear infiltration and

extensive necrosis (Huizinga *et al*., 1979). Marked pathological changes were limited to the gills on the 36th day infected fishes; the gill of infected fish was found haemorrhage globe (HG) formation on the filament in the present study (Fig. 2d). Thus, other changes found in reveals that hyperplasia of so-called undifferentiated epithelial cells (Adams and Nowak, 2003) that can fuse secondary lamellae. Occasionally, fusion of the secondary lamellae entraps amoebae in interlamellar vesicles in some tissues in this study. Therefore, one explanation for the mucous cell hyperplasia on the gill surface (Adams and Nowak, 2003) concurrent with excessive mucus production is also observed, as is an infiltration of leucocytes within the central venous sinus adjacent to AGD lesions and lesions themselves (Adams and Nowak, 2003). Leucocytes also migrate into interlamellar vesicles containing amoebae and presumably destroy the pathogen (Adams and Nowak, 2001). As a consequence of lamellar fusion, there is a reduction in the surface area of the respiratory epithelium.

Despite this, during the present study the fish inflammation of the gill filament and hemorrhage globe was associated with the development of severe necrosis on the 36th day in infected fishes (Fig. 2d). Previous researchers suggest that *C. carpio* artificially infected with viremia-associated aneaki-byo in combination with *A. hydrophila* respiratory epithelia and interlamellar epithelia were found separated due to edematous dilation of the choroidal membrane (Miyazaki *et al*., 2001). In *I. punctatus* infected with *A. hydrophila* the gill lymphocytes were found to accumulate in intercellular spaces with slight hyperplasia and spongiosis in lamellar epithelium (Grizzle and Kiryu, 1993). Therefore, some other alterations exists in large mouth bass (*Micropterus salmoides*) infected with *A. hydrophilathe* gills had severe haemorrhage and necrosis exposing the supporting cartilage, loss of functional integrity of gill tissue, diffuse edema, profuse haemorrhage, mononuclear infiltration and extensive necrosis (Huizinga *et al*., 1979). In *S. salar* vaccinated with *V. anguillarum* the multifocal fusion appeared in the skin resulting in hyperplasia, synechiae and became necrotic. Hyperplastic epithelial cells had an increase in the respiratory diffusion distance due to addition of hypertrophic epithelial cells (Morrison *et al*., 2001). *Sardinops sagax*, infected with epizootic gill foci, developed edema, proliferation of chloride cells, hypertrophy and hyperplasia, focal to multifocal inflammatory lesions in the secondary lamellae (Whittington *et al*., 1997). Laglar *et al*. (1963) reported that gaseous exchange and other solutes between blood and water take place in the gill lamellae. Based on the above findings, it is tempting to suggest that bacteria might provide a gills source of major infectious organ.

Therefore, gill is one of the main sites for antigen uptake and retention (Dalmo *et al*., 1997). In the present study gills of treated fishes on the 6th day showed regenerative responses like proliferation of granular cells and thickening of the secondary lamellae (Fig. 2e). Histological lesions were observed on 12th day there was only a moderate hypertrophy (MHT) of lamellar epithelium (Fig. 2f). We found that some of regenerative response was related to fibrosis and infiltration of the leucocytes (neutrophils and monocytes) was seen on the 24th day (Fig. 2g). On the 36th day of treatment the regenerative response of late treated fish revealed regeneration of fibrosis and infiltration of leucocytes (neutrophils and monocytes) (Fig. 2h). The current study also showed that the altered histopathological changes after herbal treatment were recovered.

Therefore, in agreement with other authors we

have shown that Abutbul *et al*. (2004) in tilapia fed with a diet containing ethyl acetate extract of *Rosmarinus officinalis* leaf powder and Fujiki *et al*. (1994) in carp administered with the fraction II of *Undaria pinnatifida*. They also found that the administration of this fraction 6 and 3 days prior to intraperitoneal challenge with *E. tarda* significantly increased the survival rate (Fujiki *et al*., 1994). The disease resistance against *A. hydrophila* was enhanced in *L. rohita* fed with 0.5% of Achyranthes (Rao *et al*., 2006). The methanolic herbal extracts of *S. trilobatum*, *Andrographis paniculata* and *Psoralea corylifolia* helped to increase the survival and growth and reduced the bacterial load even in the shrimp, *Penaeus monodon* post larvae (Citarasu *et al*., 2003). Even though, the fractions enhanced the nonspecific immune mechanisms, the hexane soluble fraction was more protective than the water soluble fraction when it was administered twice. This enhancement in nonspecific immune responses and the protection by hexane soluble fraction might be due to the presence of the compound, Sobatum. Sobatum, a partially purified component of *S. trilobatum* was obtained from the petroleum ether/ethyl acetate (75 : 25) extractable portion, which was identified as b-sitosterol by comparison with an authentic sample and proved to be an anticancer agent by *in vitro* and *in vivo* experiments (Govindan *et al*., 2004). This phytosterol has shown to be effective in stimulating various components of the immune system at mammalian level (Bouic *et al*., 1996). Administration of water or hexane soluble fraction enhanced some of the nonspecific immune parameters and disease resistance against *A. hydrophilain* tilapia. Scores of medicinal herbs have already been tested and used with good results in the control of fish diseases (Campbell *et al*., 1998).

In India, herbs have long been used for promotion of health, prevention and treatment of diseases (Evans, 1994). *Ocimum sanctum* L. has been claimed to be valuable against a wide variety of diseases. Indian Materia Medica describes the use of the plant in the treatment of a number of ailments like bronchitis, rheumatism, pyrexia (Nadkarni, 1976). Although a few study on the immunomodulatory effect of *O. sanctum* have been reported for various animal species (Singh and Majumdar, 1997; Sadekar *et al*., 1998). The leaf of *O. sanctum* has been shown to contain water soluble phenolic compounds, and various other constituents such as eugenol, methyl eugenol and caryophyllene (Chopra *et al*., 1956) that might act as a potential immunostimulant. However, the active principle responsible for the restorative property observed in the present study. Similar immunostimulatory effect has been observed in *O. mossambicus* administered with azadirachtin, a triterpenoid derived from the seed kernel of *Azadirachta indica* (Logambal and Michael, 1997) and with other plant extracts in mice (Ray *et al*., 1996), rats (Sen *et al*., 1993) and broiler chicken (Sadekar *et al*., 1998). The prospects of using natural products including plant extracts in the treatment of motile aermonad septicemia (Harikrishnan *et al*., 2003), epizootic ulcerative syndrome (Campbell *et al*., 1998) and some parasitic diseases like myxobolasis, trichodinosis, gyrodactylosis, argulosis, etc., in farmed tropical freshwater fish has been reported recently (Dey, 1997).

Similar possible findings were also observed immunostimulants originated from plant, animal and bacterial sources. Bath treatment of *Catla catla* spawn with 1% crude extract of neem, garlic and turmeric (1:1:1) (Dey and Chandra, 1995), and oral treatment of *Labeo rohita rohu* with *Catharanthus roseus* extract (Thuy *et al*., 2002) and yellowtail *Seriola quinqueradiata* with glycyrrhizin (Edahiro *et al*., 1990) enhanced immune response and dis-

ease resistance against experimental infection with *Streptococci*. Similarly different plant sources were reported to enhance the antibody response in fishes. For example, intraperitoneal administration of leaf extract of *Acalypha indica*, *Phyllanthus niruri* and seed kernel of *Azadirachta indica* has enhanced the antibody response in tilapia (*Oreochromis mossambicus*) against sheep red blood cells (SRBC) (Hemapriya *et al*., 1997).

Nimbidin exerts a significant anti-ulcer effect; it can completely inhibit the growth of *Mycobacterium tuberculosis in vitro* and nimbolide also shows antibacterial activity against *Staphylococcus aureus* and *S. coagulase* (Rojanapo *et al*., 1985). Azadirach tin is a possible future drug as an immunostimulant or an adjuvant in vaccination that stimulates and enhances primary and secondary immune response intilapia, *Oreochromis mossambicus* (Logambal and Michael, 2001). They were identified by histopalogical studies indicate that when infected with *A. hydrophila*, after herbal treatment promotes tissue regeneration in gold fish *C. auratus*. It has been assumed that there is possibility for herbal treatment of *A. hydrophila* infection may be a worth while alternative to standard antibiotic treatments and investigations into the effect of herbal extract treatment of other fish diseases may reveal further uses for this strategy. Therefore, further research is needed to provide adequate evidence of the cause of diseases and their treatment.

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References

Abutbul, S., Golan-Goldhirsh, A., Barazani, O. and Zilberg, D.: Use of *Rosmarinus officinalis* as a treatment against *Streptococcus iniae* in tilapia (*Oreochromis* sp.). Aquaculture, 238: 97-105, 2004.

Adams, M.B. and Nowak, B.F.: Amoebic gill disease: sequential pathology in cultured Atlantic salmon, *Salmo salar* L. J. Fish Dis., 26: 601-614, 2003.

Adams, M.B. and Nowak, B.F.: Distribution and structure of lesions in the gills of Atlantic salmon, *Salmo salar* L., affected with amoebic gill disease. J. Fish Dis., 24: 535-542, 2001.

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

Anto, R.J., Kuttan, G., Dinesh Babu, K.V., Rajasekharan, K.N. and Kuttan, R.: Anti-inflammatory activity of natural and synthetic curcuminoids. Pharm. Pharmacol. Commun., 4: 103-105, 1998.

Bell, S.C., Patel, S., Hales, M.W., Kirwan, P.H. and Drife, J.O.: Immunochemical detection and characterization of pregnancy-associated endometrial α1-and α2-globulins secreted by human endometrium and decidua. J. Reprod. Fertil., 74: 261-270, 1985.

Bhadauria, M., Jadon, A., Sharma, A. and Shukla, S.: Effect of propriety herbal formulation against chronic carbon tetrachloride induced hepatotoxicity. Indian J. Exp. Biol., 40: 1254-1259, 2002.

Bouic, P.J.D., Etsebeth, S., Liebenberg, R.W., Albrecht, C.F., Pegel, K. and Van Jaarsveld, P.P.: Beta-sitosterol and Beta-sitosterol glucoside stimulate human peripheral blood lymphocyte proliferation: implications for their use as an immunomodulatory vitamin combination. Int. J. Immunopharmacol., 18: 693-700, 1996.

Branson, E.J. and Diaz-Munoz, D.N.: Description of a new disease condition occurring in farmed coho salmon, *Oncorhynchus kisutch* (Walbaum), in South America. J. Fish Dis., 14: 147-156, 1991.

Campbell, R.E., Lilley, J.H. and Richards, R. H.: The use of natural products in the treatment of EUS (Epizootic Ulcerative Syndrome), Kane A S and Poynton S L (eds.), 114, Proceedings of the International Symposium on Aquatic Animal Health. Baltimore, U.S.A, 1998.

Candan, A.A.: A study on the histopathology of *Aeromonas hydrophila* infectious of rainbow trout (*Salmo gairdneri* R.) kept under experimental conditions and the effect of chloramphenicol. J. Aquat. Prod., 4: 5-20, 1990.

Chopra, R.N., Nayer, S.L. and Chopra, I.C.: *Glossary of Indian medical plants*. New Delhi: Council of Scientific and Industrial Research, 1956.

Citarasu, T., Venkatramalingam, K., Babu, M.M., Sekar, R.R.J. and Petermarian, M.: Influence of the antibacterial herbs, *Solanum trilobatum, Andrographis paniculata* and *Psoralea corylifolia* on the survival, growth and bacterial load of *Penaeus monodon* post larvae. Aquacult. Int., 11: 583-595, 2003.

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

Crespo, S. and Sala, R.: Utraltrastructural alterations of the dogfish (*Scyliorhinus canicula*) gill filament related to experimental aquatcz inc pollution Dis. Aquat.. Org., 1: 99-104, 1986.

Dalmo, R., Ingerbrightsen, K. and Bogwald, J.: Non-specific defence-mechanisum in fish, with particular reference to the reticuloendothelial system (RES). J. Fish Dis., 20: 241-273, 1997.

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

Dey, R.K. and Chandra, S.: Preliminary studies to raise disease resistant seed (fry) of Indian major carp, *Catla catla* (Ham.) through herbal treatment of spawn. Fish Chimes, 23: 5, 1995.

Dey, R.K.: On the use of herbal materials for managing diseases and health conditions of fish during sustainable aquaculture practices. Abstract, National workshop on fish and shellfish health management, CIFA, Bhubaneswar, India, 1997.

Donta, S.T. and Haddow, A.P.: Cytotoxic activity of *Aeromonas hydrophila*. Infect. Immun., 21: 989-993, 1978.

Edahiro, T., Hamoguchi, M. and Kusuda, R.: Effect of Glycyrrhizine against Streptococcal infection of young yellowtail, *Seriola quinqueradiata*. Suisan Zoshoku, 38: 239-43, 1990.

Ellis, A.E., Roberts, R.J. and Tytler, P.: The anatomy and physiology of teleosts, Roberts R J (ed), pp. 13-55, Fish Pathology. 2nd edition,

Bailliere Tindall, Londan, 1989.

Evans, M.: A Guide to Herbal Remedies. Orient paper back, Delhi, 9-12, 1994.

Fanta, E., Rios, F.S., Romao, S., Vianna, A.C.C. and Freiberger, S.: Histopathology of the fish *Corydoras paleatus* contaminated with sublethal levels of organophosphorus in water and food. Ecotoxicol. Environ. Safety, 54: 119-130, 2003.

Fujiki, K., Matsuyama, H. and Yano, T.: Protective effect of sodium alginates against bacterial infection in common carp, *Cyprinus carpio* L. J. Fish Dis., 17: 349-355, 1994.

Gernhofer, M., Pawet, M., Schramm, M., Muller, E. and Triebskorn, R.: Ultrastructural biomarkers as tools to characterize the health status of fish in contaminated streams. J. Aquat. Ecosystem, Stress and Recov., 8: 241-260, 2001.

Govindan, S., Viswanathan, S., Vijayasekaran, V. and Alagappan, R.: Further studies on the clinical efficacy of *Solanum xanthocarpum* and *Solanum trilobatum* in bronchial asthma. Phytothera. Res., 18: 805-809, 2004.

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

Gupta, S.K., Prakash, J. andSrivastava, S.: Validation of traditional claim of Tulsi, *Ocimum sanctum*Linn as a medicinal plant. Indian J. Exp. Biol., 40: 765-773, 2002.

Harikrishnan, R.: Herbal treatment for ulcerative disease induced by *Aeromonas hydrophila* in goldfish (*Carassius auratus*). PhD thesis. Bharathidasan University, Tiruchirapalli, India, 2003.

Harikrishnan, R. and Balasundaram, C.: Modern trends in *Aeromonas hydrophila* disease management with fish. Rev. Fish. Sci., 13: 281-320, 2005.

Harikrishnan, R., Balasundaran, C. and Bhuvaneswari, R.: Restorative effect of *Azadirac hta indica* aqueous leaf extract dip treatment on haematological parameter changes in *Cyprinus carpio* (L.) experimentally infected with *Aphanomyces invadans* fungus. J. Appl. Ichthyol., 21: 410-414, 2005.

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

Hemapriya, V. S., Logambal, S.M. and Michael, D.R.: Immunostimulation by leaf extracts of a few south Indian medicinal plants in *Oreochromis mossambicus* (Peters). Dev. Comp. Immunol., 21: 175-179, 1997.

Hinton, D.E. and Lauren, D.J.: Liver structural alterations accompanying chronic toxicity in fishes: potentioal biomarkers of exposure, McCarthy J F and Shugart LR (eds.), 51-65, Biomarkers of Environmental Contamination. Boca Raton, Lewis Publishers, 1990.

Hocutt, C.H. and Tilney, R.L.: Changes in gill morphology of *Oreochromis rnossambicus* subjected to heat stress. Environ. Biol. Fish., 14: 107-114, 1985.

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

Ilori, M.O., Sheteolu, A.O., Omonigbehin, E.A. and Adeneye, A.A.: Antidiarrhoeal activities of

Ocimum gratissimum (Lamiaceae). J. Diarrhoeal Dis. Res., 14: 283-285, 1996.

Iwalokun, B.A., Gbenle, G.O., Smith, S.I., Ogunledun, A., Akinsinde, K.A. and Omonigbehin, E. A.: Epidemiology of shigellosis in Lagos, Nigeria: trends in antimicrobial resistance. J. Health Popul. Nutr., 19: 183-190, 2001.

Janssen, D.B., Jan Van Der Ploeg, F.P., Kazemier, B., Terpstra, P. and Witholt, B.: Cloning of 1, 2 - Dichloroethane Degradation genes of *Xanthobacter autotrophicus* GJ10 and Expression and Sequencing of the *dhl*A Gene. J. Bact., 171: 6791- 6799, 1989.

Joseph, S.W. and Carnahan, A.: The Isolation, Identification, and Systematic of the motile *Aeromonas* species. Annual Rev. Fish Dis., 4: 315-343, 1994.

Kongtorp, R.T., Kjerstad, A., Taksdal, T., Guttvik, A. and Falk, K.: Heart and skeletal muscle inflammation in Atlantic salmon, *Salmo salar* L.: a new infectious disease. J. Fish Dis., 27: 351-358, 2004.

Kovacs-Gayer, E.: Histophathological differential diagnosis of gill changes with special regard to gill necrosis, Olah J (ed), 219-232, Fish, pathogens and environment In European polyculture, Akademiai & Ado, Budapest, 1984.

Laglar, K.F., Bardach, J.E., and. Miller , R.R.: Ichthyology. John Wiley and Sons Inc. London, 1963.

Logambal, S.M. and Michael, R.D.: Azadirachtin-An immunostimulant in *Oreochromis mossa mbicus* (Peters). Abstract, National workshop on fish and shellfish health management, CIFA, Bhubaneswar, India, 1994.

Mitra, C.R., Garg, H.S. and Pandey, G.N.: Constituents of *Azadirachta indica* 3. identification of nimbidic acid and nimbidinin from *Azadirachta indica*. Phytochemistry, 10: 857-864, 1971.

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

Miyazaki, M.: Forms and morphs of aphids, Aphids: Their biology, natural enemies and control, Minks A K and Harrewijn P (eds.), 27-50, Vol. A. Elsevier Science Publisher, Netherlands, 1987.

Morrison, R.N., Nowak, B.F. and Carson, J.: The histopathological effects of a levamisole-adjuvanted *Vibrio anguillarum* vaccine on Atlantic salmon (*Salmo salar* L.). Aquaculture, 195: 23-33, 2001.

Nadkarni, K.M.: Indian Materia Medica (3rd ed.), Popular Prakashan, Bombay, 1260-1262, 1976.

Nakamura, C.V., Nakamura, T.U., Bando, E., Fernandes, A., Melo, N., Cortezdag. and Filho, B. P.: Antibacterial activity of *Ocimum gratissmum* L. essential oil. Mem Inst Oswaldo Cruz Rio deJaneiro., 94: 675-678, 1999.

Ortuno, J., Esteban, M.A. and Meseduer, J.: High dietary intake of α-tocopherol acetate enhances the non-specific immune response of gilthead seabream (*Sparus auratus* L.). Fish Shellfish Immunol., 10: 293-307, 2000.

Pazhanisamy, K.: Studies on the impact of arsenic trioxide on a freshwater fish, *Labeo rohita* (Hamilton). Ph.D. Thesis, Annamalai University, India, 2002.

Peters, G., Hoffmann, R. and Klinger, H.:. Environmentalinduced gill disease of cultured rainbow trout (*Salmo gairdneri*). Aquaculture,

38: 105-126, 1984.

Peters, G. and Hong, D.Q.: Gill structure and blood electrolyte levels of European eels under stress, Fish and shellfish pathology, Ellis A E (ed), 183-198, Academic Press, London, 1985.

Rao, Y.V., Das, B.K., Jyotyrmayee, P. and Chakraba rti, R.: Effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. Fish Shellfish Immunol., 20: 263-273, 2006.

Rath, R.K.: Freshwater Aquaculture. Scientific Publishers, Jodhpur, India, 282-312, 1993.

Ray, A., Banerjee, B.D. and Sen, P.: Modulation of humoral and cell mediated immune responses by *Azadirachta indica* (Neem) in mice. Indian J. Exp. Biol., 34: 698-701, 1996.

Rojanapo, W., Suwanno, S., Somaree, R., Glinsukon, T. and Thebtaranonth, Y.: Screening of antioxidants from some Thai vegetables and herbs. J. Sci. Thailand., 11: 177-188, 1985.

Sadekar, R.D., Kolle, B.S. Barmase, B.S. and Desai, V.F.: Immunopotentiating effects of *Azadirachta indica* (Neem) dry leaves powder in broilers, naturally infected with IBD virus. Indian J. Exp. Biol., 36: 1151-1153, 1998.

Schwaiger, J., Wanke, R., Adam, S., Pawert, M., Honnen, W. and Triebskorn, R.: The use of histopatological indicators to evaluate contaminant-related stress in fish. J. Aquat. Ecossystem, Stress and Recovery, 6: 75-86, 1997.

Selvam, R., Subramanian, R., Gayathri, R. and Angayarkanni, N.: The anti-oxidant activity of turmeric (*Curcuma longa*). J. Ethanopha macol., 47: 59-63, 1995.

Sen, P., Maiti, P. C., Puri, S. and Ray, A.: Mechanism of antistress activity of *Ocimum sanctum* Linn, eugenol and *Tinospora malabarica* in experimental animals. Indian J. Exp. Biol., 30: 596-605, 1992.

Sen, P., Medirapta, P., Ray, A. and Puri, S.: An experimental evaluation of *Azadirachta indica* in normal and stressed rats and adaptogenic effect, Chary M S, Singh R P, Krus W, Saxena R C (eds.), Abstract, World Neem Conference. Bangalore, India, 1993.

Shao, J-Z., Liu, J. and Xiang, L.: *Aeromonas hydrophila* induced apoptosis in *Carassius auratus* lymphocytes *in vitro*. Aquaculture, 229: 11-23, 2004.

Shotts, E.B. and Rimler, R.B.: Medium for the isolation of *Aeromonas hydrophila*. App. Microbiol., 26: 550-553, 1973.

Singh, K.P., Gupta, A.S., Pendse, V.K., Mahatma, O.P., Bhandari, I.S. and Mahawar, M.M.: Experimental and clinical studies on *Tinospora coradifolia*. J. Int. Med. Res., 10: 9-12, 1999.

Singh, S. and Majumdar, D.K.: Evaluation of anti-inflammatory activity of fatty acids of *O. sanctum* fixed oil. Indian J. Exp. Biol., 35: 380-383, 1997.

Soltani, M., Mirzargar, S.S. and Abrahizadeh, H.A.: Occurrence of a motile *Aeromonas* septicaemia in the imported ornamental fish, oscar *Astronotus ocellatus*: Isolation characterization and pathogenicity. J. Fac. Med. Uni. Tehran., 53: 63-65, 1998.

Teh, S.J., Adams, S.M. and Hinton, D.E.: Histopathological biomarkers in feral freshwater fish populations exposed to different types of contaminant stress. Aquat. Toxicol., 37: 51-70, 1997.

Thophon, S., Kruatrachue, M., Upathan, E.S., Pokethitiyook, P., Sahaphong, S. and Jarikh

uan, S.: Histopathological alterations of white seabass, *Lates calcarifer* in acute and subchronic cadmium exposure. Environ. Poll., 121: 307-320, 2003.

Thuy, N.T.T., Mukherjee, S.C. and Prasad, K.P.: Studies on the immunostimulatory effect of certain plant extracts on fish. The Sixth Indian Fisheries Forum, CIFE, Mumbai, India, Abstracts AH-13: 153, 2002.

Van der Nat, J.M., Klerx, J.P., Dijk, H.V., De Silva, K.T. and Labadie, R. P.: Immuomodulatory activity of an aqueous extract of *Azadirachta indica* stem bark. J. Ethanopharmacol., 19: 125-131, 1987.

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

Whittington, R.J., Jones, J.B., Hine, P.M. and Hyatt, A.D.: Epizootic mortality in the pilchard *Sardinops sagax* neopilchardus in Australia and New Zealand in 1995. I. Pathology and epizootiology. Dis. Aquat. Org., 28: 1-16, 1997.

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

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