J. Indian Fish Assoc., 35: 45 - 50, 2008

IMMNUNOMODULATORY AND CYTOTOXIC EFFTECTS OF AQUEOUS LEAF EXTRACTS OF CERTAIN PLANTS ON COMMON CARP, *CYPRINUS CARPIO*

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ABSTRACT

Effect of aqueous leaf extracts of *Catheranthus roseus, Calotropis gigantium* and *Datura stromoneum* on common carp, *Cyprinus carpio* were investigated. *C. carpio* were separately fed with 1 and 2% aqueous extracts of these three plant leaves for a period of seven days. In 1% *Catheranthus roseus* of leaf extract fed group no significant tissue level changes were recorded. One and 2% of other two species fed treated group showed mild to severe necrotic and cellular changes in liver, kidney and spleen. Immunologically, significant rise in antibody titre and respiratory burst activity was recorded for 1% *Catheranthus roseus* fed group.

Key words: ***Cyprinus carpio, Catheranthus roseus, Calotropis gigantium, Datura stromoneum, E.tarda.***

INTRODUCTION

The role of plants or their products as antimicrobial, antineoplastic, cardiovascular and insecticide have already been established in many living systems (Al-Sereiti *et al.*, 1999; Sashikumar *et al.*, 2003).In aquaculture due to emergence of antibiotic resistant strains of bacteria much attention has been paid for boosting the immune system of fish by using a wide range of chemicals, drugs, microbial products and plant products. However, among several immunostimulants use of herbal products have attracted a lot of focus in combating infectious diseases now-a-days (Campbell *et al.*, 1998; Chakraborty and Chattopadhyay, 1998; Dey, 1997).

A number of herbal materials including *Ocimum sanctum, Acalpha indica, Phyllanthus niruli, Azadiracta indica* and *Piper bitle* have been evaluated for treatment of various infectious diseases. However, most of the studies were directed on the *in-vitro* antibacterial potentialities of the plant extracts. Informations regarding their effect on cellular as well as immune system remained scanty. The present investigation evaluated the effect of aqueous leaf extracts of commonly available plants *viz. Catheranthus roseus, Calotropis gigantium* and *Datura stromoneum* on the immune system and vital organs of common carp.

MATERIALS AND METHODS

Fish

Common carp, *Cyprinus carpio* of average body weight (13 ± 2 g) were used in the present experiment.

Preparation of Plant Extracts

Three plants, namely *Catheranthus roseus* (Apocynaceae), *Calotropis gigantium* (Ascepidaceae) and *Datura stromoneum* (Solanaceae) were used for evaluation. The crude aqueous extracts from different plants were prepared as per the method of Logambal *et al.*, (2000) with slight modifications. The leaves were thoroughly washed with tap water followed by immersion in alcohol for about 10 minutes. About 550 g of leaves were macerated in 150 ml of distilled water in a clean mortar and pestle and the whole products were centrifuged at 10,000 rpm for 10 min. The supernatant was then filtered through Whatman No. 1 filter papers and stored at 4°C till further use.

Preparation of Feed

Laboratory prepared control feed containing 35% protein was used. The same feed mixed with 1 and 2% of leaf extracts and fed @ 3% body weight twice daily.

Experimental Design

Fish was maintained in 1000 l plastic pools with constant aeration and provision of daily water exchange. During the course of investigation the physico-chemical parameters of water were examined before and after feeding. In six groups (3 plant extracts X 2 concentrations) in duplicate, the fish were fed with experimental diet while the control group was fed with control diet.

Histopathology

After 7 days of the feeding 5 fish from each group were sacrificed, kidney, liver and spleen were collected in neutral buffered formalin and processed for histopathological examination following routine procedure (Roberts, 2001).

NBT assay

The NBT assay was conducted as per the protocol of Sigma (USA, 1978). About 0.1 ml of freshly prepared 0.02% NBT solution (Sigma, USA) was mixed with 0.1 ml of blood and 15 µl of stimulant (Sigma, USA) in a pyrogen free eppendorf tube. The mixture was incubated for 10 min at 37°C followed by another 10 min at 26°C. After incubation a thick smear was made over a glass slide and stained with Wright stain (Sigma, USA) for 15 min followed by 30 seconds treatment with distilled water and the slides were then dried, observed under oil immersion microscope. Five fish in each group were used to calculate the percentage of NBT + cells. The NBT + cells containing formazan granules were counted.

Antibody response

Ten fish from each group were immunized with attenuated *E. tarda* strain (obtained from Aquatic Animal Health Management Division, CIFE, Mumbai, India), after 7 days of feed treatment. Hundred microlitre of *E. tarda* bacterin ($1x10^6$ CFU/ml) was intraperitoneally injected into fish followed by two booster doses in 36 hours intervals. After 10 days of immunisation, the fish were bled through cardiac puncture, serum separated and stored at -20^0 C until further use.

The agglutination test was conducted in 'U' shaped microtitre plates. About 100 µl of antibody was serially two fold diluted in phosphate buffer saline (pH 7.2) and then added with equal volume of *E. tarda* antigen ($1x10^6$ CFU/ml). The plates were incubated at $28^{\circ}C$ in humid chamber for 2 hours and observed for agglutination. The antibody titre was calculated as the reciprocal of highest dilution showing complete agglutination reaction.

RESULTS

During the present investigation neither any significant difference in feed consumption efficacy in fish nor any difference in physico-chemical parameters of water before and after feed treatment were recorded in any group.

No mortality in any of the feed treated group was recorded. However, the liver tissue of fish fed with 1 and 2% of *D. stromoneum* extracts showed degenerative to necrotic changes with fragmentation and condensation of nuclei (Fig. 1), while hyperplasic changes were noted in kidney (Fig. 2). The fish fed with *C. gigantium* showed increased cellularity of liver, kidney and spleen (Fig. 3). However, in case of *C. roseus* at 1% feed level fed group the liver tissue showed minimum changes (Fig. 4) while increased cellurality was recorded in kidney (Fig 5) and moderate hyperplasia of spleen at 2% level.

Significantly increase in NBT + cells (respiratory burst activity) was recorded in 1% followed by 2% *C. roseus* feed treated group. However, in other groups higher respiratory burst activity was recorded at 1% level than 2% fed groups (Fig. 6). In all the treated groups increased antibody response was recorded with all the leaf extracts fed at 1% level. Significantly higher antibody titre (1:64) was recorded with C. roseus at 1% level followed by *C. gigantium* and *D. stromoneum* (Fig. 7).

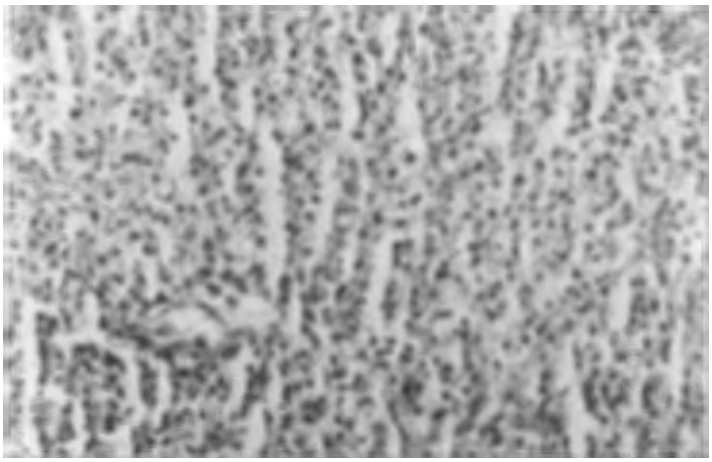

Fig. 1 Photograph of liver of fish fed with 1% *Datura stromoneum* showing

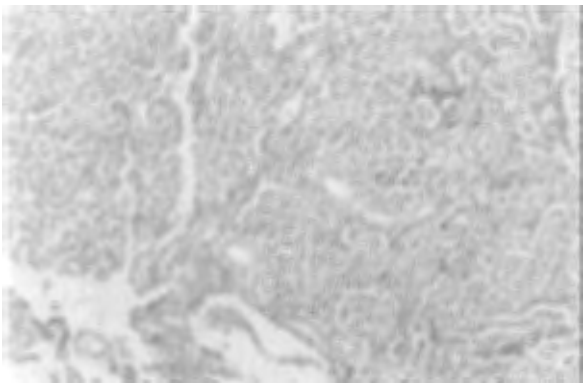

Fig. 2 Photograph of kidney tissue showing hyperplastic changes when fed with 2% extracts of *Datura stromoneum*

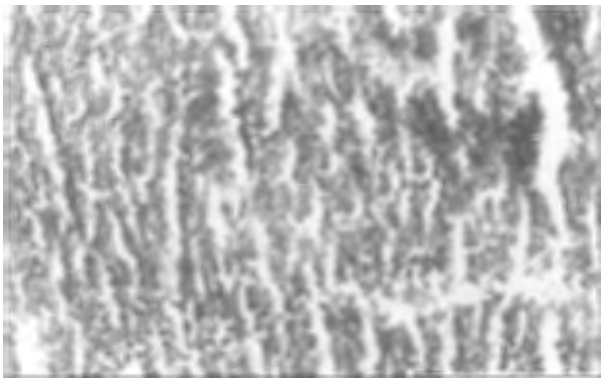

Fig. 3 Photograph of spleen showing hyperplastic changes in feed with 2% extracts of *Calotropis gigantium*

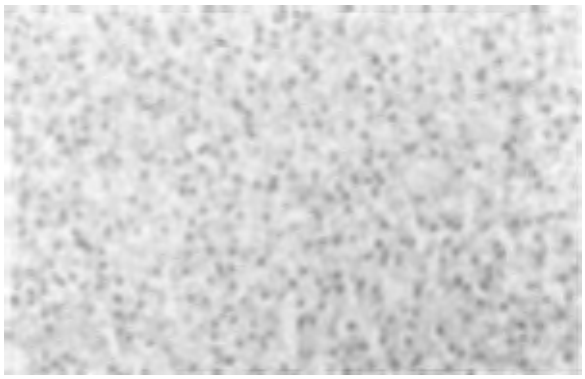

Fig. 4 Photograph of liver of fish fed 2% extract of *Catheranthus roseus* leaf showing vacuolated hepatic cells

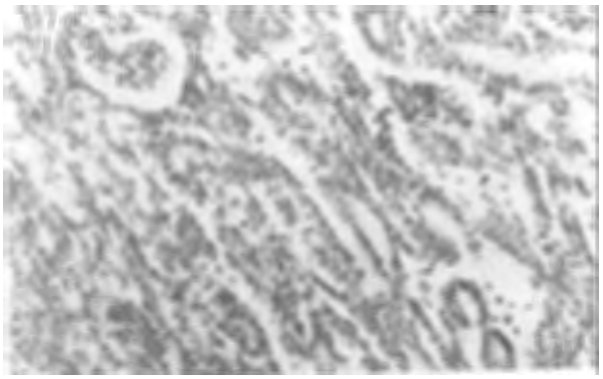

Fig. 5 Photograph of kidney of fish fed with 1% extracts of *Catheranthus roseus* showing increased cellularity

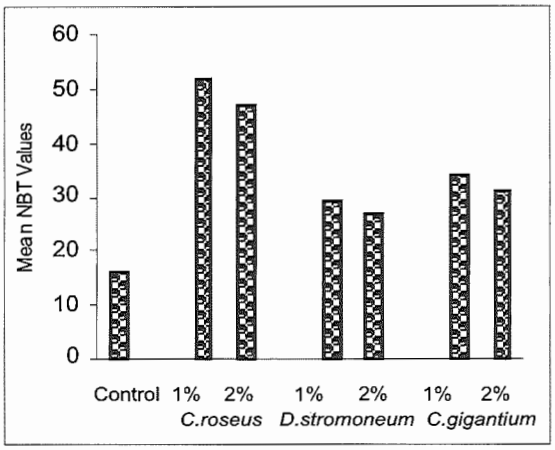

Fig.6 The Mean NBT values in *Cyprinus carpio* fed with different concentrations of leaf erxtracts of *C. roseus,D.stromoneum* and *C. gigantium*

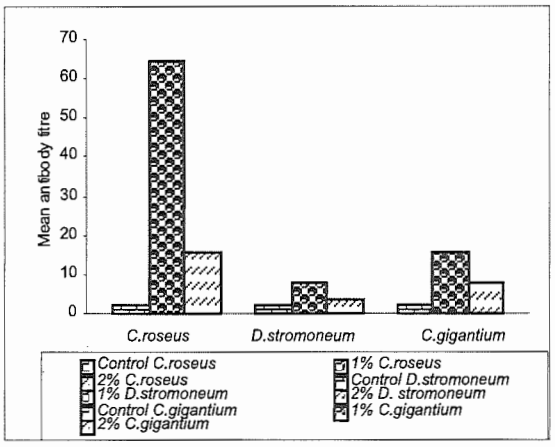

Fig. 7 The mean antibody titre against *E.tarda* in *Cyprinus carpio* fed with different concentrations of leaf extracts of *C. roseus, D. stromoneum* and *C. gigantium*

DISCUSSION

During last few decades aquaculture has changed from extensive type to intensive type and this often deteriorated the pond environment leading to the outbreak of infectious diseases.

The control and prevention of these diseases are mainly achieved by using a wide range of chemicals and drugs. The regular use of these drugs leads to morphological deformities of host, development of resistivity among pathogens and public health hazards (Baticados and Paclibare, 1992). To overcome these problems, several immunostimulants *viz. lipopolysaccharides, chitin, chitosan, glucan*, quaternary ammonium compounds, vitamins, hormones and plant extracts are now being used in aquaculture practices. The present investigation show the immmunopotentiating ability of *C. roseus, C. gigantium* and *D. stromoneum* extracts. Both the respiratory burst activity and antibody response were enhanced by all the three plant extracts fed at 1% level. The findings went in accordance with the findings of Venkatesan *et al.*, (2001), Michael, (2001) and Logambal *et al.*, (2000). However, both the responses declined when the plant extracts were fed at 2% level. This might be due to initial stimulation of immune system followed by adverse and/ or suppression of immune response from drug-induced tissue damage. The leaf extracts of *C. roseus* at 1% level significantly enhanced the immune response in comparison to all other treatments. Several researchers like Campbell *et al.*, (1998); Chakraborty and Chattopadyay, (1998) and Dey, (1997) have worked on various plant extracts and herbal products using different fish species and have advocated on the potentiality of herbal extracts in controlling infectious diseases in fish. It was also seen that the leaf extracts of *C. roseus* at 1% level can be safely advocated to be added in the fish feed to be protected against microbial infection. However, systematic work and data on various species of fish are very limited especially in relation to the dose and toxicity level. Considering the enormous biodiversity of medicinal plants available in the country more attention should be given in fixing their dose, duration of application, effect on immune system and toxicity before recommending

them for use in aquaculture.

ACKNOWLEDGMENTS

The authors are thankful to the Director, CIFE, Mumbai, India for his encouragement and support in carrying out the research work.

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