



ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2016; 2(2): 361-364
www.allresearchjournal.com
Received: 22-12-2015
Accepted: 24-01-2016

Dr. S Subeenabegum
Assistant Professor
In Zoology, Alagappa
university, Karaikudi,
Tamil Nadu, India.

Dr. PS Navaraj
Principal, Annai Fatima
College of Arts and Science,
Madurai, Tamil Nadu, India.

Dietary supplement of mixture of medicinal plant leaf extracts on immune response of fresh water fish *Mystus keletius*

Dr. S Subeenabegum, Dr. PS Navaraj

Abstract

The present work aimed to estimate the dietary supplement of mixture of medicinal plant leaf extract of *Solanum trilobatum* and *O.sanctum* as well as the individual effect on immune response of fresh water fish *Mystus keletius*. Non-specific immune assays such as phagocytic activity, serum antiprotease activity and serum myeloperoxidase activity were analysed in freshwater cat fish *Mystus keletius*. The blood sample was collected for every seven days after the final feeding. Levels of phagocytic activity, serum antiprotease activity and serum myeloperoxidase activity were increased in the group of fish fed with supplemented diet than the group fed with control diet. This increase was based on the concentration of plant extracts mixture and duration of supplemented diet. This increase was more in fish supplemented with *Solanum trilobatum* leaf extract than in fish treated with *Ocimum sanctum* leaf extract but lesser than the fish fed with mixture of aqueous leaf extracts of both in 1:1 ratio. Highest level of phagocytic activity, serum antiprotease activity and serum myeloperoxidase activity were confirmed when the fish fed with 1% of mixture of aqueous extracts of *Solanum trilobatum* and *O.sanctum* in the 4th week of the experiment. The synergistic effect of medicinal plant aqueous leaf extracts mixture on immunostimulatory activity of fish is highlighted.

Keywords: Immunostimulatory, aqueous leaf extract, phagocytic activity, serum antiprotease activity, serum myeloperoxidase activity, *Mystus keletius*.

1. Introduction

Aquaculture is a fast developing industry contributing fish protein with minerals such as zinc, magnesium, sodium to the consumers (Ravenhalt, 1982; and Barlas, 1986) [28, 2]. Intensive fish farming creates a highly stressful environment for the fish with consequent suppression of the immune response resulting in increased susceptibility to diseases (Sakai *et al.*, 1991; Yano *et al.*, 1991; and Austin and Austin, 1999) [31, 33, 1]. The non-availability of commercial vaccines for many diseases, particularly in developing countries, poses a threat for the prevention of diseases. Current trends in the use of medicinal plant extracts as an alternative to the drugs, chemicals and antibiotics in controlling fish diseases is growing because plant extracts, in contrast to vaccines, enhance the innate (or nonspecific) immune response (Sakai 1999) [32]. Many herbal plants found to inhibit the bacterial pathogens and activate the immunity (Chansue *et al.*, 2000; and Dugenci *et al.*, 2003) [3, 8] at a low concentration and hence its use was very cost effective (Lipton, 2009) [17]. The immunostimulatory effect of medicinal plants in fish was observed; *Ocimum sanctum* in *Cyprinus carpio* (Pavaraj *et al.*, 2011) [25]; *Solanum trilobatum* in *Oreochromis mssambicus* (Divyagnaneswari *et al.*, 2007) [7]; The effect of plant extract mixture on fish immune response has been documented; mixture of Chinese herbs via., *Rheum officinale*, *Andrographis paniculata*, *Isatis indigotica*, *Lonicera japonica* on carp fish (Chen *et al.* 2003) [4]; *Viscus album*, *Urtica dioica* and *Zingiber officinale* on Rainbow trout (Dugenci *et al.*, 2003) [8]; The oral administration of plant extracts as a feed supplement has enhanced the immune responses in fish. Several findings such as *Cynodon dactylon* in *Cyprinus carpio* (Kaleeswaran *et al.*, 2010) [14]; Phagocytosis has been recognized as an important activity in the host's defense against invading microorganisms (MacArthur *et al.*, 1985; and Olivier *et al.*, 1986) [18, 24]. Fish were showing enhanced phagocytic activity due to various immunostimulants such as *Ocimum sanctum* in *Cyprinus carpio* (Pavaraj *et al.*, 2011) [25]; *Solanum trilobatum* in *Cyprinus carpio* (Durga Devi and Balasubramanian, 2009) [9];

Correspondence

Dr. S Subeenabegum
Assistant Professor
In Zoology, Alagappa
university, Karaikudi,
Tamil Nadu, India.

and the mixed herbal diet from *Punica granatum*, *Dalmatian chrysanthemum*, *Chrysanthemum cinerariaefolium* in marine fin fish olive flounder (Harikrishnan *et al.*, 2010) [12]. Antiproteases are the one of the components of non-specific immunity of the vertebrates. It was observed in *Catla catla* fed with *A. aspera* seed diet for four weeks (Rao and Chakrabarti, 2004; and Rao and Babu, 2009) [29, 30] and *Cynodon dactylon* in *Catla catla* (Kaleeswran *et al.*, 2011) [15]; in *L. rohita* fed with diet containing herbal ingredients (Rao *et al.*, 2004) [27]. The mixture of plant extracts has also demonstrated a good Myeloperoxidase activity in fish; Chinese herbs *Rheum officinale*, *Andrographis paniculata*, *Isatis indigotica* *Lonicera japonica* in Chinese sucker fish (Chen *et al.*, 2003) [4]; aqueous extracts of *Viscum album*, *Urtica dioica*, *Zingiber officinale* in rainbow trout (Dugenci *et al.*, 2003) [8]. These findings suggest that medicinal plant leaf extracts could be an alternative to the chemotherapeutics in aquaculture when it was used as supplement in the diet. The main objective of this study was to determine the synergistic effect of plant extracts added to the diet on nonspecific immune response of *Mystus keletius*

2. Materials and methods

2.1 Maintenance of fishes

Clinically active fish purchased from local ponds around Madurai were acclimated to laboratory conditions in daily renewed fresh water for fifteen days. Healthy fishes were fed ad libitum with a balanced diet prepared in the laboratory before the commencement of the experiment.

2.2 Preparation of Diets

The balanced diet prepared by adding fishmeal, soyabean meal, ricebran, wheat flour, vitamins and minerals in appropriate proportion. The nine experimental diets namely E1, E2, E3, E4, E5, E6, E7, E8, & E9 were prepared by adding the appropriate concentration of chosen medicinal plant aqueous extracts as follows. The diet E1-E3 were supplemented with aqueous extract of *S. trilobatum*, the diet E4-E6 were supplemented with aqueous extract of *O. sanctum*, the diet E7-E9 were supplemented of *S. trilobatum* and *O. sanctum* mixture of 0.01%, 0.1%, 1.0% respectively.

2.3 Preparation of Aqueous Extract (AE)

The extraction was done by following the method of Natarajan *et al* (2005) [22] with minor modifications. The mixture of the plant extracts of *S.trilobatum* and *O.sanctum* were prepared in equal proportion (1:1). This mixture was compared with the immunostimulatory efficacy of individual extract of *S.trilobatum* and *O.sanctum*

2.4 Serum Collection

A day after the final feeding, blood samples were obtained from the common cardinal vein of randomly chosen five fish anesthetized with 100 mg tricaine methane sulfate (MS-222) by using a 1 ml heparinized syringe for every 7 days of the experiment in each tank. Blood samples from each fish group were collected with a tuberculin syringe and stored in serological tubes. The separated serum was then stored in Eppendorf tubes at -20 °C.

2.5 Phagocytic activity

The phagocytes ratio was calculated by using the formula (Michael *et al.*, 1998) [20]

$$\text{Phagocytic index (\%)} = \frac{\text{Phagocytic leukocyte number}}{\text{Observed total leukocyte number}} \times 100$$

2.6 Antiprotease activity the percentage of trypsin inhibition was calculated as described by Rao and Chakrabarti (2004) [29]:

% of Trypsin inhibition =

$$\frac{\text{Trypsin blank OD (A1)} - \text{Sample OD (A2)}}{\text{Trypsin blank OD (A1)}}$$

2.7 Serum Myeloperoxidase activity

Serum myeloperoxidase activity in fish is measured by the method of Quade and Roth (1977) with partial modification (Sahoo *et al.*, 2005).

3. Statistics

Mean, Standard Deviation, ANOVA tests, Tukey's Multi comparison test were performed in this study by using the SPSS software package. Significance was also set at 5% level.

4. Results

The phagocytic activity increases were 11.96 %, 29.35% and 35.14% in the first week; 30.0%, 35.56% and 56.67 % in the second week; 53.77%, 58.07% and 78.48 % in the third week; and 63.34%, 75.56% and 108.89% in the fourth week after the fish fed with 0.01%, 0.1% and 1.0% aqueous plant mixture extracts (*S. trilobatum* and *O. sanctum*) respectively. Thus, plants extract mixture elicited a high level phagocytic activity in the third and fourth week of the experiment. Moreover, the highest dose, 1.0% plant extract showed a better effect than other doses of the plants extract mixture in *M. keletius* (Fig-1) This study confirms that plants extract mixture has elevated more phagocytic activity than an individual extract.

The serum antiprotease activity was elevated maximum in the third and fourth week than in the first, second week of the experiment. Further, the highest dose, 1% has shown a better effect than other doses of the plants extract mixture (*Solanum trilobatum* and *Ocimum sanctum*) in *M. keletius* (Fig.2.). This confirms the high immunostimulatory function of plants extract mixture (*Solanum trilobatum* and *Ocimum sanctum*) in *M. keletius*.

The serum myeloperoxidase activity is elevated to a higher level in 1.0% of the plants extract mixture (*S.trilobatum* and *O. sanctum*) supplemented fish. (Figs.3).

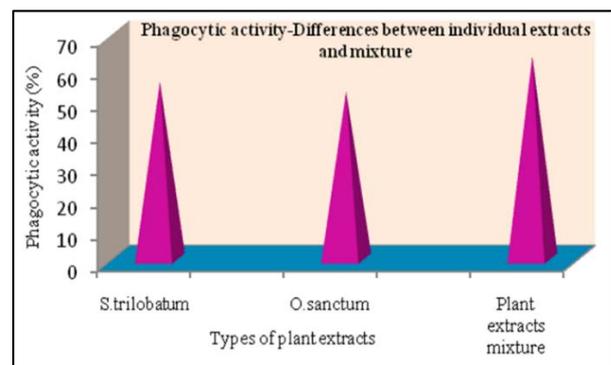


Fig 1: Graphical presentation showing Phagocytic activity in *Mystus keletius* (Differences between individual extracts and mixture)

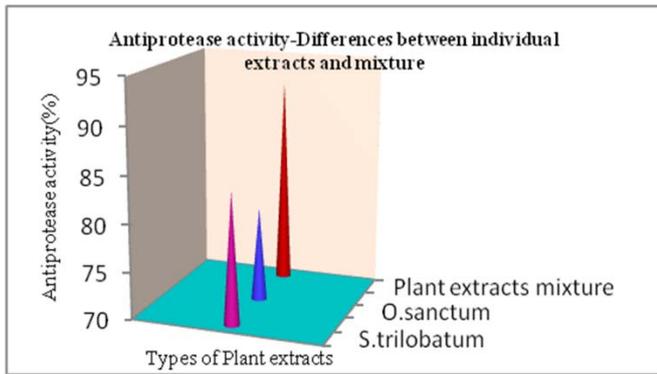


Fig 2: Graphical presentation showing Serum antiprotease activity in *Mystus keletius* (Differences between individual extracts and mixture)

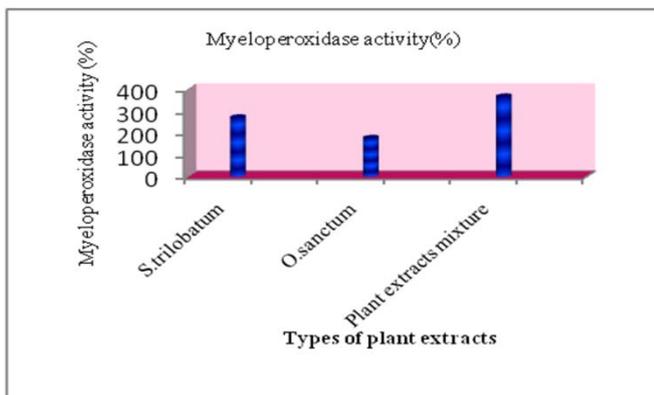


Fig 3: Graphical presentation showing Serum myeloperoxidase activity in *Mystus keletius* (Differences between individual extracts and mixture)

5. Discussion

The present study focuses the effect of *S. trilobatum* and *O. sanctum* plant aqueous leaf extracts alone or in combination administered orally on the phagocytic activity, serum antiprotease activity, serum myeloperoxidase activity in *M. keletius*. Hitherto, the oral administration 1% of the plant extract elevates the phagocytic activity in the fish in the third and fourth week of the experiment. This observation corroborates with the findings of the phagocytic activity, in hybrid tilapia fed with a diet supplemented with garlic for 2 weeks (Ndong and Fall, 2011) [23]; in tilapia fed with a diet supplemented with *Allium sativum* for 4 weeks (Govind *et al.*, 2012) [11]. Further, the highest dose of plant extracts mixture, *S. trilobatum* and *O. sanctum*, 1% has demonstrated a better phagocytic activity in *M. keletius*. Phagocytosis is recognized as the vital innate immune response of fish (McArthur *et al.*, 1985) [18] that provides a protection to the fish against pathogens (Rijkers, 1982) [30]. Moreover, the enhancement of the phagocytic activity in fish may be due to the first level defense against pathogen (Manjrekar *et al.*, 2000) [19]. The mixture of *S. trilobatum* and *O. sanctum* in 1:1 ratio of the highest dose of 1% has demonstrated a better enhancement of the serum antiprotease activity in *M. keletius* in this study. This is in agreement with the works in rainbow trout (*Oncorhynchus mykiss*) fed with a diet supplemented with 0.5%, 1.0% and 2.0% of lupin (*Lupinus perennis*) mango (*Mangifera indica*) and stinging nettle (*Urtica dioica*) (Elham, 2010) [10]. The enhancement of the serum antiprotease activity observed in this study may be due to the inhibiting the function of extracellular enzymes in fish and by restricting the replication of microbial pathogens without

untoward toxicity to the host (James *et al.*, 1999) [13]. Compared to the control, the serum myeloperoxidase activity has shown a substantial increase via, 203.92% in *S. trilobatum*, 192.45 % in *O. sanctum* and 375 % of the plants extract mixture administered orally to the fish, *M. keletius*. This corroborates with the finding of 200 % increase in catfish, *Clarius batrachus* fed with β -1,3 glucan (Kumari and Sahoo, 2006b), 180% increase in *O. mossambicus* fed with 1% *E. alba* (Christybapita *et al.*, 2007) [5], 80% increase in *Catla catla* fed with *Cynodon dactylon* (Kaleeswaran *et al.*, 2011) [15]. This may be due to the synergism of phyto components of the plant extract in enhancing the serum myeloperoxidase activity in *Mystus keletius*.

6. Conclusion

The plant leaf extract in combination rather than alone used in this study considerably has enhanced the non-specific immunity in *Mystus keletius*. In addition the underlying molecular mechanism beside the isolation and characterization of the active compounds from those medicinal plants require more study.

7. Acknowledgement

The researchers are thankful to the management of both colleges, Yadava College, Madurai and M.S.S. Wafk Board College, Madurai for their strong support in carrying out this research.

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