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Therapeutic effects of Neem (*Azadirachta indica*) leaf extract on fin and tail rot diseased fish *Anabas testudineus* (Bloch,1972)

Research Article

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Abstract

Bacterial infections significantly hinder aquaculture practices, leading to substantial economic losses. Among these, fin and tail rot disease—caused by various Gram-negative bacteria—is one of the most prevalent and challenging diseases to treat, particularly in its advanced stages. This study explored the therapeutic efficacy of *Azadirachta indica* (neem) for managing tail-rot in *Anabas testudineus*. Neem leaf extract was administered at two concentrations- 0.1 g/2 L and 0.4 g/2 L for treatment durations of 7 and 14 days, respectively. The results revealed remarkable recovery in the infected fish, with complete healing of fin and tail lesions, as well as ulcerations, following neem treatment. Hematological analysis further demonstrated a significant improvement in total RBC and WBC counts compared with the control group, highlighting the potential of neem in promoting fish health. The findings of this study underscore the effectiveness of neem leaf extract as a natural, eco-friendly remedy for combating bacterial infections in aquaculture. Its successful application offers a promising alternative to synthetic antibiotics, which aligns with sustainable aquaculture practices. The significance of this study lies in its contribution to the development of herbal-based therapies that are not only effective but also environmentally safe. However, further research is essential to fully elucidate the molecular mechanisms underlying neem's therapeutic action, optimize its dosage, and evaluate its long-term impact on fish health and aquaculture productivity.

Keywords: *Azadirachta indica, Anabas testudineus,* Haematological parameters, Bacterial disease.

Introduction

Aquaculture is one of the fastest growing food production practices to meet major demand of animal proteins (1). Outbreaks caused by infectious diseases impose major downfall in fish production causing great economic loss annually (2,3). Fish infections are caused by various infectious agents such as bacteria, virus, fungi and parasites, among which bacteria cause significant threat and others serve as secondary opportunistic invaders affecting fish immunity thereby hampering fish production worldwide (4). The extensive use of antimicrobial agents and antibiotics to control infections have sometimes led to the production of more resistant pathogens and also indirectly affecting human health (5). Herbal extracts from some plants have medicinal role that can be used to treat diseased fishes (6). *Azadirachta indica* (Neem) is well known for traditional Indian medicine which carry antibacterial, antiviral and insecticidal properties (7). Neem contains various chemical components such as nimbin, azadirachtin, meliantroil etc, containing antiviral, antibacterial and antifungal attributes (8). Blood parameters act as a useful tool for assessment of pathological and physiological changes (9). Significant variation in hematological parameters helps to show health status of fish indicating parasitic contaminations and other abnormalities (10). The objective of the present study is to examine the therapeutic attributes of neem in the treatment of diseased fishes and analyse the blood parameters with respect to the control group.

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Materials and methods

Collection of experimental materials

Azadirachta indica A. Juss. (neem) leaves were collected from nearby area of Guwahati, Assam, India. Collected neem leaves were thoroughly washed in tap water. 20 grams of neem leaves were then sun dried and ground well. The powdered neem extract was then put into a clean vessel for further use. A total of 14 *Anabas testudineus* fishes (4 healthy and 8 diseased fishes) of about length 14-16cm and weight about 45-55 grams were collected. After primary morphological screening, fish diseases were found to be bacterial; as they are showing rotting of fins, tails along with breakage of some areas of fins and; haemorrhage and ulcer in body parts. The fishes were brought to the laboratory in well aerated condition and introduced to aquarium (44 x 30 x 30 cm) filled with tap water. All the fishes were fed with commercial fish food (pellets) two times a day.

Experimental design

A total of 14 fishes were taken in the experiment into following experimental groups- Group 1- It consist of 3 healthy diseased free fishes (Disease free control group). Group 2- diseased fishes were further divided into two groups having 4 diseased fishes in each which were treated with 0.1g/2L and 0.4g/2L concentrations of neem leaf extract separately for 7 days and 14 days respectively. The neem extract solution was prepared by dissolving the powdered neem leaves in water at the specified concentrations (0.1 g/2 L and 0.4 g/2L), ensuring thorough mixing for uniform dispersion. The diseased fishes were placed in separate aquariums filled with the neem-treated water. The neem extract was applied externally by immersing the infected fishes in the treated water. The solution was refreshed every 24 hours to maintain its potency throughout the treatment period. The fishes were observed daily for signs of healing, including the reduction of lesions, ulcers, and tail-rot symptoms.

Haematological parameter analysis

For haematological parameters like; total RBC and WBC count, blood cells were counted using Neubauer's Haemocytometer following the protocol used by Harikrishnan et al 2003 (11). Differential Leucocyte Count (DLC) was carried out by preparing thin blood smears followed by staining the slides with Giemsa stain.

Statistical analysis

One way ANOVA was used to analyse the statistical significance between the control and treated fishes by using SPSS software.

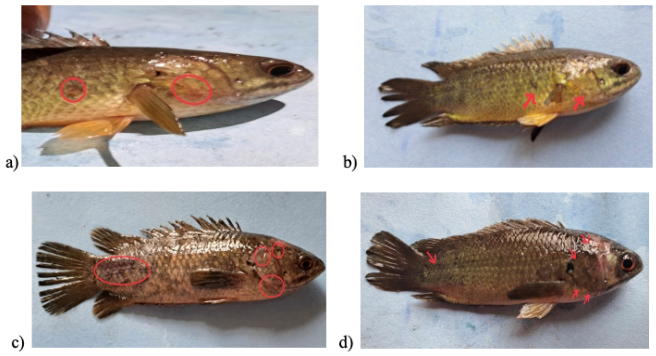
Results and Discussion

It was found that the fishes belonging to the diseased free control group behaved actively with normal swimming behaviour while the diseased fishes were observed with fast movement. After the diseased fishes were treated with neem leaf extract, they behaved almost similar to the control group. Due to frequent exposure to treated groups, the treated fishes thus showed a sign of relaxation.

Gradual improvement along with wound healing and new skin formation was observed after treatment with different doses of neem extract in the diseased fishes. At 0.1g/2L concentration of neem leaf extract, the lesions on the head region became more distinctly prominent and started to heal slowly during 7 days experimentation. At 0.4g/2L concentration after 14 days of exposure, complete healing was seen where the scars disappeared completely and no lesion marks were visible (Fig 1).

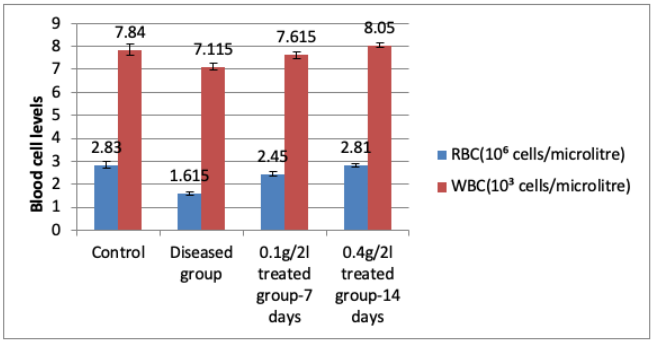
Haematological parameters of fishes are found to be highly sensitive to various biological and environmental factors such as age, weight, nutrition, food quality, water quality, stress and pathogens thereby providing information for detection of various diseases (12). The pathophysiological condition of fishes is well reflected by determining its haematological parameters which serve as indicators of various diseases, environmental stress, exposure to toxic metals and pollutants, therefore used for clinical diagnosis of fish physiology (13). In the present study, a change in the haematological parameter values have been remarked in both diseased and treated fish groups as compared to the control fish group. Increase in the mean total RBC count of the diseased fishes was noticed after the treatment with neem leaf extract to 2.45± 0.08×10^6 µL and 2.81 ± 0.08×10^6µL from 1.615±0.075×10^6cells/µL at concentrations 0.1g/2L and 0.4g/2L for 7 days and 14 days respectively. Similarly mean total WBC count was increased after the treatment in the diseased fishes from 7.115±0.165×10^3 cells/µL to 7.615±0.145×10^3 cells/µL and 8.05±0.08×10^3 cells/µL at 0.1g/2L for 7 days and 0.4g/2L for 14 days (Fig 2). The overall, WBC count showed reduction in diseased control group and WBC count returned to normal value in healthy control fish after treatment with neem leaf extract.

Figure 1:



a) Diseased *Anabas testudineus* before treatment with 0.1g/2L concentration neem leaf extract. b) Result showing the effects of neem leaf extract on ulcer disease in *Anabas testudineus*at the concentration of 0.1g/2L. The lesions near to the pectoral fin started to heal after the treatment with neem within 7 days. c) Diseased *Anabas testudineus* before treatment with 0.4g/2L concentration neem leaf extract. d) Result showing the effects of neem leaf extract on ulcer disease in *Anabas testudineus* at the concentration of 0.4g/2L. (It was observed that the ulcer and other lesions on the fish body healed completely within 14 days of treatment with 0.4g/2L concentration of neem leaf extract).

Figure 2: **Graph showing total RBC and WBC count of control, diseased and treated group. Significance at P<0.05 observed after 14 days neem treatment at 0.4g/2L concentration**



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From the DLC, it has been found that there is a decrease in the number of lymphocytes and basophils after treatment with neem compared to diseased fish. This trend has been seen approaching towards control group of fish. It was observed that the numbers of neutrophils and monocytes increased significantly in the treated groups as compared to the diseased group. (Table 1).

Table 1: DLC of diseased fishes and fishes exposed to neem extract

Parameters	Control	Diseased group	0.1/2L treated group	0.4g/2L treated group
DLC % (n=300)				
Neutrophils	21.13±0.17	10.9±0.27	18.3±0.21*	18.8±0.05*
Monocyte	13.1±0.08	9.1±0.31	11.7±0.32*	12.7±0.13*
Eosinophil	4.9±0.12	4.2±0.26	4.4±0.16	4.62±0.25
Basophil	3.67±0.35	4.6±0.37	3.34±0.23	3.51±0.30
Lymphocyte	57.2±0.28	71.2±0.36	62.26±0.24*	60.37±0.21*

Values are mean ± SEM, * Significant at P <0.05 level

Table 2: Results of one-way ANOVA between and within different groups of fishes

Sources of variation	Degree of freedom	Sum of Squares	Mean Sum of Squares	F value	p-value
Between groups	3	240.3	80.1	0.15	0.071
Within groups	56	29906	534		
Total	59	30146.3			

One-way ANOVA analysis results (Table 2) showed that as P-value is greater than the threshold value so it can be concluded that there is a significant difference between the groups of the diseased and treated fish.

People in India, mostly living in rural areas extensively use *Azadirachta indica*, (neem) to treat various wounds, ring worm, acne, skin diseases, ulcers etc (14). Various medicinal plants and herbs have great potential in controlling fish diseases (4,15). The application of neem leaf extract is successful in curing lesions and ulcers occurring in diseased fishes (6). The increase in the total RBC and WBC count post-treatment indicates enhanced immunity and recovery in neem-treated fish (3), who also observed a significant improvement in haematological parameters and lesion healing in *Anabas testudineus* treated with neem leaf powder in their study on hole-in-the-head (HITH) disease. Similarly, Alam et al. (6) reported that herbal extracts, including neem, significantly reduced the mortality rate and improved the overall health condition of diseased fish, highlighting its potential as a bio-antimicrobial agent.

Furthermore, the decrease in lymphocyte and basophil count and the increase in neutrophils and monocytes post-treatment in the present study indicate an active immune response, which is consistent with the observations of Harikrishnan et al. (11) during herbal treatment for *Aeromonas hydrophila* infection in common carp. Their study revealed that herbal therapy could modulate fish immune responses, promoting better resistance against bacterial infections. Comparing the present findings with previous literatures, the neem extract concentrations used in this study (0.1g/2L and 0.4g/2L) showed remarkable efficacy in treating fin and tail rot disease. The recovery rate and haematological improvements observed in the present study are in agreement with earlier research (8), which signified the efficacy of neem leaf juice as a bio-safe anesthetic and healing agent in *Anabas testudineus* and *Channa punctata*. The consistency of the present results with those of prior studies reinforces the therapeutic potential of neem in aquaculture. Thus, it can be concluded that, neem has the ability to control and minimise diseases caused by bacteria like ulcers and lesions on the body surface, fin or tail rot diseases etc and will be very beneficial to the aquaculture industry in preventing various infections as it is healthy, cost effective and free of side effects.

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