Effect of aqueous extract of *Polygonum minus* leaf on the immunity and survival of African catfish (*Clarias gariepinus*)

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ABSTRACT

Objective: To establish immunomodulatory potential of aqueous extract of *Polygonum minus* (*P. minus*) leaves, the haematological effects and lysozyme activity of aqueous extract of *P. minus* leaf on *Clarias gariepinus* was studied.

Methods: The fish were grouped and administered with the *P. minus* aqueous leaf extract intraperitoneally. Blood and serum samples were collected from each group and examined for various blood parameters. The turbidimetric assay for lysozyme activity using *M. luteus* and the survival rate of fish against *A. hydrophila* was carried out.

Results: There was no significant impact (*P* >0.05) on white blood cell count, red blood cell count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and lysozyme activity was significantly (*P*<0.05) higher than control. The serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase levels were significantly lower than control (*P*<0.05). The highest dose group showed longest survival.

Conclusions: From this study, it is considered that the aqueous extract of *P. minus* can be used as an immunostimulant in African catfish, but continuous administration may require maintaining the protection.

KEYWORDS

Haematological effect, African catfish, *Polygonum minus*, Aqueous extract, *Clarias gariepinus*, Lysozyme activity, Immunomodulatory activity

1. Introduction

*Polygonum* s.l. (*Polygonaceae*) is cosmopolitan genus of about 170 species, which are mainly distributed in the temperate northern regions with few species in the tropical and subtropical regions[1]. It is originated from Southeast Asia countries namely Malaysia, Thailand, Vietnam and Indonesia. It grows wild in damp areas near the river banks, ditches and lakes. It survives well on cool and hilly area[2]. This plant is locally named as ‘kesum’ in Malay and often consumed as ulam (raw medicinal plants consumed with daily meals) for preventive health care. In the Malaysian Traditional Medicine System, the decoction of kesum leaf is recommended for digestive disorders and stomach pain[3]. *Polygonum minus* (*P. minus*) has a kind of sweet and pleasant aroma and is commonly used amongst the Malaysian population as a flavouring ingredient in Malaysian food, Laksar[2]. *P. minus* was chosen for our research purpose mainly because of its enormous use in traditional medicine. Studies have reported that *P. minus* possess antioxidant activity[3,4], antimicrobial activity[5], antiulcer activity[6], cytotoxicity and antiviral activity[7,8].
There is no published report on immunomodulatory effect of *P. minus* leaf extract on catfish.

Use of expensive chemotherapeutic agents and antibiotics for managing various infections has been widely criticized for their pessimistic impact like residual accumulation in the tissue, development of resistance and immune-suppression, thus resulting in reduced consumer preference for fish as a food treated with antibiotics\[^9\]. Prevention of disease is much more desirable than intervention to stop and reverse the disease process once it begins.

This approach could be achieved with application of vaccines, which can enhance the specific immune response of the fish and are considered to be the most effective agents. The available commercial vaccines are highly expensive for fish farmers and they are specific against particular species\[^10\]. In contrast to vaccines, immunostimulants enhance the non-specific immune response of fish\[^11,12\]. The major components of the innate immune system are macrophages, monocytes, granulocytes and humoral elements, like lysozyme\[^13-15\]. Several biological and synthetic compounds have been shown to enhance non-specific immune system of cultivated fish\[^12,16-23\].

Hence, instead of chemotherapeutic agents and vaccines, increasing attention is being paid to the use of immunostimulants as a disease control measure in aquaculture. *Aeromonas hydrophila* (*A. hydrophila*) has been reported to cause fin rot disease in hatchery reared *Cyprinus carpio* fingerlings under temperate climatic conditions of Kashmir valley and had recommended the use of multiple antibiotics as a bath for controlling fin rot disease\[^24\]. However, as mentioned above, controlling diseases with antibiotics is not a safe procedure in aquaculture practices. There was no published report on immunomodulatory effect of *P. minus* leaf extract on catfish. Based on the observations from literature survey, it was envisaged to study the various haematological and immunomodulatory effect of the water extract of *P. minus* leaf in African catfish, *Clarias gariepinus* (*C. gariepinus*).

2. Materials and methods

2.1. Extraction of plant materials

Fresh leaves of *P. minus* were collected from the Penang region of Malaysia, air-dried at room temperature (25±2 °C) for 2 weeks, then grinded and sieved through sieve No. 20 to get uniform powder. Then 150 g of the powder was extracted (agitated in an orbital shaker at 185 r/min for 72 h) with 600 mL of distilled water in six separate conical flasks with each conical flask contains 25 g of powder. The water extracts were then filtered using muslin cloth and the filtrate was concentrated using rotary vacuum evaporator. Phytochemical screening was performed using standard procedures.

2.2. Experimental fish and husbandry

Healthy African catfish with average weight (200±25) g, were procured from fish market, Sungai Petani, Kedah Darul Aman, Malaysia. The fish were kept acclimatized for a period of 5 d at (25±2) °C in circular cement tanks (400 L) and fed with formulated catfish diet pellet once daily. Water exchange was done daily by replacing 80% of the tank water and water quality was monitored throughout the experiment.

2.3. Preparation of injection dose

The extract powder was properly weighed in two quantities such as 375 mg and 250 mg, dissolved separately in 25 mL of distilled water, filtered through 0.45 µm membrane filter and labeled as high dose (15 mg/kg) and low dose (10 mg/kg) respectively.

2.4. Experimental design

African catfish (*n*=108) were selected for the study and divided into 3 groups (A, B and C). Each group is consisting of 36 fish, which was again divided into three equal subgroups. Group A (control) was injected with distilled water. Group B and C were injected with low and high dose of plant extract respectively. Feed was withheld before blood sample were collected. Eighteen fish from each group (6 fish from each subgroup) was randomly picked for blood withdrawal at two days intervals. Blood was collected from caudal vein of catfish with the help of needle. Blood and serum samples were examined for the following parameters: total protein, albumin, globulin, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), packed cell volume (PCV), white blood cells (WBC), serum alkaline phosphatase, haemoglobin and serum lysozyme activity.

2.5. Lysozyme activity and challenge on survival of fish

A virulent strain of *A. hydrophila* and *Micrococcus luteus* received from Faculty of Biotechnology, AIMST University was used in this study. The bacterial suspension (1×10^7 CFU/mL) was prepared by using the method reported by Sahu *et al.*\[^25\] and used for the challenge experiment and lysozyme activity. The turbidimetric assay for lysozyme was carried out according to Parry *et al.*\[^26\].

Thirty African catfish were taken for this study and divided into three groups containing equal number of fish. After the administration of plant extract, fish were challenged intraperitoneally with virulent *A. hydrophila* (1×10^7 CFU/mL, 0.2 mL) on Day 2 and observed for a 10–day period for mortality.

3. Results

WBC count was higher in both the treated groups than the control but not significant (*P*>0.05) (Table 1). The RBC count increased with the administration of *P. minus*. Haemoglobin level in group C was significantly higher as compared to group A on both days, but non–significantly higher in group B on both days. MCV and MCH content was significantly higher in group B and C as compared to group A on both days (Table 1). MCHC count was significantly higher in group C on day 2 and 4 as compared to group A.
There was no significant (P>0.05) difference on both days in group B when compared to group A. There was significant (P<0.05) level of increment in PCV level in both treatment groups on both days compared to control.

The serum total protein, albumin and globulin in treated groups was not significantly (P>0.05) different from control, but the amount is higher than the control. Alkaline phosphatase level was significantly (P<0.05) higher in both treated groups compared to control. SGOT and SGPT level was significantly (P<0.05) lower in both treated groups compared to control (Table 1).

Table 1

| Parameters | Group A | Group B | Group C |
|------------|---------|---------|---------|
| RBC (10^6 cells/mm³) | 2.9840±0.24 | 2.9400±0.35 | 2.9740±0.35 |
| Hb (g/L) | 72.00±6.55 | 73.33±7.37 | 75.67±7.39 |
| MCV (fL) | 88.00±6.85 | 86.67±7.39 | 89.67±8.06 |
| MCH (pg) | 133.33±8.96 | 134.67±9.58 | 132.67±10.34 |
| MCHC (g/L) | 39.67±4.04 | 42.00±4.52 | 40.00±5.00 |
| Platelets (10^3/mm³) | 49.00±2.37 | 47.67±2.52 | 48.00±2.52 |

Even though, the significant increase (P<0.05) in RBC and haemoglobin level, non–significant increase in WBC count observed in this present study agree with the works of Banerjee and Maulic (2002), and Martins et al. (2002) who reported increases in total leucocyte count, neutrophils, lymphocytes, monocytes, RBC and haemoglobin concentration in rats when fed with garlic containing diet. Sahu (2004), Duncan and Kiesius (1996) and Salah et al. (2008)[32] observed significant (P<0.05) increase in erythrocytic count in fish fed with a diet containing β-glucan and garlic. WBCs play an important role in non specific immunity and their count can be considered as an indicator of the health status of fish.

In the present study, the level of PCV of fish in this present study, concur with that of Martins et al. (2002) and Salah et al. (2008), however, contradicts the results of Banerjee and Mailic (2002), Adelol et al. (2011) and Ugwu and Omale (2011), observed significantly decreased PCV values in the treated rats when compared with that of the control. Meanwhile, red cell indices (MCV, MCH and MCHC) on the other hand, are particularly important for the diagnosis of anemia in humans and most animals. Significant increases (P<0.05) in these red cell indices as observed in the treated fishes in this current study is not agree with the findings of Martinez et al. (2007). It is assumed that the decrease or increase of blood indices may be attributed to a defense reaction against P. minus, which occurs by stimulation of erythropoiesis.

Increase in serum protein, albumin and globulin levels are thought to be associated with a stronger immune response of fish[37]. The change in level of alkaline phosphatase, SGOT and SGPT may be due to the hepatoprotective effect of the plant.

4. Discussion

P. minus has primarily been described as antioxidant, antimicrobial, antiviral, antiulcer and cytotoxic. Hence, the present work focused on the study of immunomodulatory activity of water extract of P. minus leaf in African catfish. The percentage yield of crude water extract of P. minus leaf was 15%. The phytochemical screening of the water extract of P. minus showed the presence of reducing sugar, terpenoids, flavonoids, saponins and tannins. These phytochemical constituents may play a crucial role in the immune system stimulation and in the function of organs related to blood cell formation such as thymus, spleen, and bone marrow as reported by Jeorg and Lee[27].
reported an increased protection against fish bacterial infection, which correlated with an increment in serum lysozyme levels, phagocytic activity and bactericidal activity of head kidney leucocytes. So this results concerning lysozyme activity support the observations that humoral factors may enhance phagocytosis in fish.

Administration of medicinal plant extracts has been reported to enhance the immune ability of the fishes. In the present study, African catfish injected with water extract of P. minus at 10 and 15 mg/kg displayed an increase in resistance to A. hydrophila after 2 to 10 days of challenge. The best survival rate [90.48±2.749%] was observed in group C. The relative percentage survivability of group B was 36.36 and group C was 81.82. Therefore, the water extracts of P. minus at 15 mg/kg or less showed positive effects on preventing A. hydrophila infections.

In conclusion, the present results suggest that African catfish which received aqueous extract of P. minus at a dose of 15 mg/kg or less exhibited a significant stimulatory effect on the non-specific immune mechanisms tested, and disease resistance against A. hydrophila. Further purification of the active compounds and their evaluation may substantially improve quality as well as their usage in the culture system. It is considered that aqueous extract of P. minus can be used as an immunostimulant in African catfish, but continuous administration may be necessary to maintain the protective response. Also further investigation on the immunostimulatory effect of this plant preparation when administered along with feed (which is the preferred route of administration in the field) for disease prevention in aquaculture is warranted.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Herbal immunostimulants are substances which may render fishes more resistant to infectious diseases, by stimulating phagocytic cells as well as complement lysozyme and antibody responses of fish. In this research, the effects of P. minus aqueous leaf extract was studied on its effects on the haematooimmunological indices and survival rate of African catfish (C. gariepinus).

Research frontiers

The present study illustrates the effects of various doses of aqueous extract of P. minus leaf on the immunity responses, haematology indices and survival rate of African catfish (C. gariepinus).

Related reports

P. minus is a perennial herb of the Polygonaceae family. There is a lack of evidence in support of P. minus for any clinical indication. P. minus has been studied as antioxidant and antimicrobial in refrigerated duck meatballs. Antioxidant effect of P. minus may help to improve the immune system in the body.

Innovations and breakthroughs

P. minus is locally known as kesum, has antioxidant effects thus may be a potential source of natural antioxidants. This study investigated the potency of Polygonum minus as immunostimulant in fish. The objective of this study was to determine whether P. minus is sufficiently potent to protect immune system of fish.

Applications

P. minus has been studied as antioxidant and antimicrobial in refrigerated duck meatballs. P. minus is likely safe when eaten in normal amounts in the diet. It must be cautious when consuming the leaves of Polygonum plants, due to their oxalic acid content which may cause kidney stone formation.

Peer review

This is a research work in which authors have illustrated haematoo-immunological effects P. minus on African catfish (C. gariepinus). They indicated that dose of 15 or 10 mg/kg of P. minus aqueous leaf extract had significant stimulatory effects on the non-specific immune mechanisms and disease resistance in A. hydrophila.

References

[1] Heywood VH, Brummitt RK, Culham A, Seberg O. Flowering plant families of the world. Kew, UK: Royal Botanic Gardens; 2007.
[2] Qader SW, Abdulla MA, Chua LS, Hamdan S. Potential bioactive property of Polygonum minus Huds (kesum) review. Sci Res Essays 2012; 7: 90–93.
[3] Vimala S, Rohana S, Rashih AA, Juliza M. Antioxidant evaluation in Malaysian medicinal plant: Persicaria minor (Huds). leaf. Sci J Med Clin Trials 2012; doi: 10.7237/sjmct/215.
[4] Sumazian Y, Syahida A, Hakiman M, Maziah M. Antioxidant activities, flavonoids, ascorbic acid and phenolic contents of Malaysian vegetables. J Med Plant Res 2010; 4: 881–890.
[5] Johnny L, Yusuf UK, Nulit R. The effect of herbal plant extracts on the growth and sporulation of Colletotrichum gloeosporioides. J Appl Biosci 2010; 34: 2218–2224.
[6] Wasmam S, Mahmood AA, Salehuddin H, Zahra AA, Salnah I. Cytoprotective activities of Polygonum minus aqueous leaf extract on ethanol–induced gastric ulcer in rats. J Med Plant Res 2010; 4: 2658–2665.
[7] Qader SW, Abdulla MA, Chua LS, Najim N, Zain MM, Hamdan S. Antioxidant, total phenolic content and cytotoxicity evaluation of
selected Malaysian plants. *Molecules* 2011; 16: 3433–3443.

[8] Ali AM, Mackeen MM, El–Sharkawy SH, Abdul Hamid J, Ismail NH, Ahmad F, et al. Antiviral and cytotoxic activities of some plants used in Malaysian indigenous medicine. *Pertanika J Trop Agri Sci* 1996; 19: 129–136.

[9] Anderson DP. Immunostimulants, adjuvants, and vaccine carriers in fish: applications to aquaculture. *Ann Rev Fish Dis* 1992; 2: 281–307.

[10] Raa J, Rorstad G, Engstad RE, Roberston B. The use of immunostimulants to increase resistance of aquatic organisms to microbial infections. In: Shariff IM, Subasinghe RP, Arthur JR, editors. *Diseases in Asian aquaculture*. Manila, Philippines: Asian Fisheries Society; 1992, p. 39–50.

[11] Galeotti M. Some aspects of the application of immunostimulants and a critical review of methods for their evaluation. *J Appl Ichthyol* 1998; 14: 189–199.

[12] Sakai M. Current research status of fish immunostimulants. *Immunopharmacol Immunol* 1992; 14: (7): 87–91.

[13] Goetz FW, Iliev DB, McCauley LA, Liarte CQ, Tort LB, Planas JV, et al. Analysis of genes isolated from lipopolysaccharide of live and dead probiotic cells in tilapia *Oreochromis mossambicus* macrophages. *Mol Immunol* 2004; 41: 1199–1210.

[14] Hardie LJ, Fletcher TC, Secomes CJ. The effect of dietary vitamin C on the immune response of Atlantic salmon (*Salmo salar*). *Aquatculature* 1991; 95: 201–214.

[15] Siwicki AK. Immunostimulating influence of levamisole on nonspecific immunity in carp (*Cyprinus carpio*). *Dev Comp Immunol* 1999; 13: 87–91.

[16] Goetz FW, Iliev DB, McCauley LA, Liarte CQ, Tort LB, Planas JV, et al. Analysis of genes isolated from lipopolysaccharide of rainbow trout (*Oncorhynchus mykiss*) macrophages. *Mol Immunol* 2004; 41: 1199–1210.

[17] Hardie LJ, Fletcher TC, Secomes CJ. The effect of dietary vitamin C on the immune response of Atlantic salmon (*Salmo salar*). *Aquatculature* 1991; 95: 201–214.

[18] Siwicki A. Immunomodulating activity of levamisole in spawners carp (*Cyprinus carpio* L.). *J Fish Biol* 1987; 31: 245–246.

[19] Thompson I, White A, Fletcher TC, Houdihan DF, Secomes CJ. The effect of stress on the immune responses of Atlantic salmon (*Salmo salar* L.) fed diets containing different amounts of vitamin C. *Aquatculature* 1993; 114: 1–18.

[20] Shan BE, Yoshida Y, Sugira T, Yamashita U. Stimulating activity of Chinese medicinal herbs on human lymphocytes in *vitro*. *Int J Immunopharmacol* 1999; 21: 149–159.

[21] Cao LZ, Lin ZB. Regulatory effect of *Ganoderma lucidum* polysaccharides on cytotoxic T–lymphocytes induced by dendritic cells in *vitro*. *Acta Pharmacol Sin* 2003; 24: 312–326.

[22] Lin ZB, Zhang HN. Anti–tumor and immunoregulatory activities of *Ganoderma lucidum* and its possible mechanisms. *Acta Pharmacol Sin* 2004; 25: 1387–1395.

[23] Soosean C, Mariimuthu K, Sudhakaran S, Xavier R. Effect of mangosteen (*Garcinia mangostana*) extracts as a feed additive on growth and hematological parameters of African catfish (*Clarias gariepinus*) fingerlings. *Ear Rev Med Pharmacol Sci* 2010; 14: 605–611.

[24] Hussain SA, Samoon MH, Najar AM, Balkh MH, Rashid R, Occurrence of fin rot disease in common carp (*Cyprinus carpio*) in Kashmir. *J Vet Public Health* 2005; 3: 79–81.

[25] Sahu S, Das BK, Mishra BK, Pradhan J, Sarangi N. Effect of *Allium sativum* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. *J Appl Ichthyol* 2007; 23: 80–86.

[26] Parry RM Jr, Chandan RC, Shahani KM. A rapid and sensitive assay of muramidase. *Proc Soc Exp Biol Med* 1965; 119: 384–386.

[27] Jeorg HG, Lee YW. Protective effect of daily sulfide on N–nitrosodimethylamine– induced immunosuppression in mice. *Cancer Let* 1998; 11: 73–79.

[28] Iranloye BO. Effect of chronic garlic feeding on some haematological parameters. *Afr J Biomed Res* 2002; 5: 81–82.

[29] Martins ML, Moraes FR, Miyazaki DM, Brum CD, Onaka EM, Fenerick JJ, et al. Alternative treatment for *Anacanthorus pendelatus* (Monogenea: Dactylogyridae) infection in cultivated pacu, *Piaractus mesopotamicus* (Osteichthyes: Characidae) in Brazil and its haematological effects. *Parasite* 2002; 9: 175–180.

[30] Sahu S. Antibacterial activity of plant extracts on fish microbial pathogens [dissertation]. Kausalyaganga, Bhubaneswar: CIFA; 2004.

[31] Duncan PL, Klesiuu PH. Dietary immunostimulants enhance nonspecific immune responses in channel catfish but not resistance to *Edwardsiella ictaluri*. *J Aquat Anim Health* 1996; 8: 241–248.

[32] Salah MA, Nashwa MA, Mohamed FM, editors. 8th Inter Symposium on Tilapia in Aquaculture; 2008 Oct 12–14; Cairo, Egypt. Egypt: The Central Laboratory for Aquaculture Research; 2008.

[33] Banerjee SK, Maulik SK. Effect of garlic on cardiovascular disorders: a review. *Nurt J* 2002; 1: 4.

[34] Adebolu TT, Adeeoye OO, Oyetayo VO. Effect of garlic (*Allium sativum*) on *Salmonella typhi* infection, gastrointestinal flora and hematological parameters of albino rats. *Afr J Biotechnol* 2011; 10: 6804–6808.

[35] Emmanuel UC, James O. Comparative effects of aqueous garlic (*Allium sativum*) and onion (*Allium cepa*) extracts on some haematological and lipid indices of rats. *Ann Rev Res Biol* 2011; 1: 37–44.

[36] Corzo–Martinez M, Corzo N, Villamil M. Biological properties of onions and garlic. *Trends Food Sci Technol* 2007; 18: 609–625.

[37] Wiegertjes GF, Ster DJ, Parmentier HK, Van Muiswinkel WB. Immunogenetics of disease resistance in fish: a comparative approach. *Dev Comp Immunol* 1996; 20: 365–381.

[38] Engstad RE, Robertson B, Frivold E. Yeast glucan induces increase in lysozyme and complement–mediated haemolytic activity in Atlantic salmon blood. *Fish Shellfish Immunol* 1992; 2: 287–297.

[39] Taoka Y, Maeda H, Jo JY, Kim SM, Park S, Yoshikawa T, et al. Use of live and dead probiotic cells in tilapia *Oreochromis niloticus*. *Fish Sci* 2006; 72: 755–766.

[40] Jeney G, Galeotti M, Volpatti D, Jeney Z, Anderson DP. Prevention of stress in rainbow trout (*Oncorhynchus mykiss*) fed diets containing different doses of glucan. *Aquaculture* 1997; 154: 1–15.

[41] Roberson B, Rostadt G, Engstad R, Raa J. Enhancement of non–specific disease resistance in Atlantics salmon, *Salmo salar L.*, by a glucan from *Saccharomyces cerevisiae* cell walls. *J Fish Dis* 1990; 13: 391–400.

[42] Yin G, Ardó L, Thompson KD, Adams A, Jeney Z, Jeney G. Chinese herbs (*Astragalus radix* and *Ganoderma lucidum*) enhance immune response of carp, *Cyprinus carpio*, and protection against *Aeromonas hydrophila*. *Fish Shellfish Immunol* 2009; 26: 140–145.