

EFFECT OF MILK PLANT VITISVINIFERA EXTRACT ON NONSPECIFIC IMMUNITY OF RAINBOW TROUT (ONCORHYNCHUSMYKISS)

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ABSTRACT

Use of medicinal plants as compounds stimulating and strengthening the immune system of fish has been considered in recent years. However, there is a very little and inadequate knowledge in this area, unfortunately. The aim of this study was to investigate the impact of leaves and twigs extracts of *Vitisvinifera* plant on non-specific immunity of rainbow trout (*Oncorhynchusmykiss*). In this study, changes in the level of immunoglobulins IgG, total complement, lysozyme and peroxidase of fish fed by leaves and twigs of *Vitisvinifera* plant at doses of 100, 400 and 800 mg of drug per kilogram of commercial food for 28 days were examined. Changes in levels of immunoglobulin (IgM), total complement, and lysozyme activity in plasma of fish fed by nutrient supplements were not significant compared to the control group ($p < 0.05$). Peroxidase activity level in the plasma of fish fed by supplements of leaves and twigs of *Vitisvinifera* shows significant increase compared with experimental groups ($p < 0.05$). The results of this study suggest a positive impact of leaves and twigs of *Vitisvinifera* plant in relative increase in the immune system of fish.

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INTRODUCTION

The important role of immune system in maintaining the aquatic animal health and ensuring their survival and growth during the period of breeding and rearing has caused researchers to use a variety of stimulating chemical and natural compounds to strengthen the immune system. In the past decades, wide range of studies has been conducted regarding the use of medicinal plants in laboratory scale to strengthen the immune system of laboratory animals. The obtained results of these studies show a positive role of many of these medical plants and herbs in strengthening the immune system of animals [1].

For example, a study conducted on the stimulating role of medical plants of *Thymus vulgaris* and geranium *Plargvnyvm* in strengthening the immune system of laboratory animals indicate that the positive effect of these medicines in strengthening of their immune system [2]. Jafarian et al (2002) showed that the use of extract of medicinal plant of arveneh in the diet of laboratory animals can strengthen and improve the cellular and humoral immune system. Use of medicinal plants is one of the new approaches of using these compounds in pharmacology to treat and cope with viral infections [3,4], bacterial infections [5], fungal infections [1] and even preventing the spread protozoan parasites [6]. The use of medicinal plants as anti-fungal compounds [7] and anti-bacterial compounds, and as compounds stimulating the immune system [8] has old history to enhance the immune system of the fish. However, many of the known species of plants have adverse effects on consumers. For example, *Achilleatalagonica* Boiss plant and *A. tenuifolia* Lam plant from the family of yarrow plant, as one of the most important medicinal plants, are extremely toxic and deadly to artemia [7]. Milk thistle plant as member of Chicory and with scientific name of *silybummariamum*, and English name of Milk thistle has *silymarin* complex that has extraordinary medicinal properties and its role in strengthening the immune system has been proven in laboratory animals [1,7]. However, the operating mechanism of milk thistle extract as an immune system stimulant has not been described well, and its effect on aquatics has not been studied so far. The aim of this study was investigate the impact of milk thistle extract on the immune system of rainbow trout (*Oncorhynchusmykiss*).

MATERIALS AND METHODS

One hundred and twenty rainbow trout fish that their appearance was healthy (85.5 ± 15 g) were transferred to the laboratory of breeding and rearing of fish. Fish were kept in 12 tanks of 1000 liters equipped with aeration with designing system of semi-closed circuit with 10% replacement of water in the distribution day for 15 days so that they can fully adapt themselves to laboratory conditions. During this time, the fish was fed by commercial diet. The experiment of impact of leaves and twigs of *Vitisvinifera* plant on indicators of non-acquired immunity of rainbow trout over 28 days and in a completely randomized was designed with four treatments, control group fish and fish fed by different doses of leaf and twig of *Vitisvinifera* plant, and each treatment with three replications. Food was supplied weekly and freshly by adding powdered supplement of leaves and twigs of *Vitisvinifera* in 100, 400 and 800 mg per Kg of food with powder of commercial food. After the beginning of the experiment, 3 fish were selected randomly from each tank (in total 9 fish from each treatment) on 14 and 28 days of fishing and after the anesthetizing by extract of clove powder (1: 5000) from their caudal vein and using syringes covered with EDTA, their blood was collected. After centrifugation of blood samples, the plasma was isolated in a centrifuge device with the power of 6000 g for 15 minutes at 4 °C and it was stored in the freezer of -78 °C until the final tests. In measuring of peroxidase activity level of plasma, 15 mL plasma was diluted with 35 ml of HBSS buffer free from magnesium and calcium. Then, 50 ml of solution (TMB) and 5 mM of hydrogen peroxide was added so that solution turns blue, then after 2 minutes, by adding 50 ml of sulfuric acid, the colored reaction was stopped and the color of solution was changed to light yellow. In the next stage, the absorbance was measured at wavelength of 450 nm and after measuring by absorbance of the standard solution, result is expressed in terms of international unit of ml. Measurement of complement of CH50 was done by kit prepared from the company of Tehran Bahar Afshan and based on Radial Immuno Diffusion method. The activity level of Lysozyme was also measured by turbidity test and suspension of *Mvramydaz Micrococcus* and lysodeikticus enzyme. The turbidity level was also measured at a wavelength of 670. The immunoglobulin IgM of plasma was also measured by kit prepared from Tehran Bahar Afshan Company and Hitachi autoanalyzer.

Statistical analysis

Statistical analysis was done by using MINITAB 13 software and tables were drawn using EXCEL 2003 software. Statistical analysis was performed by ANOVA. In addition, the significant level of means was done by Tukey test at the 95%.

RESULTS

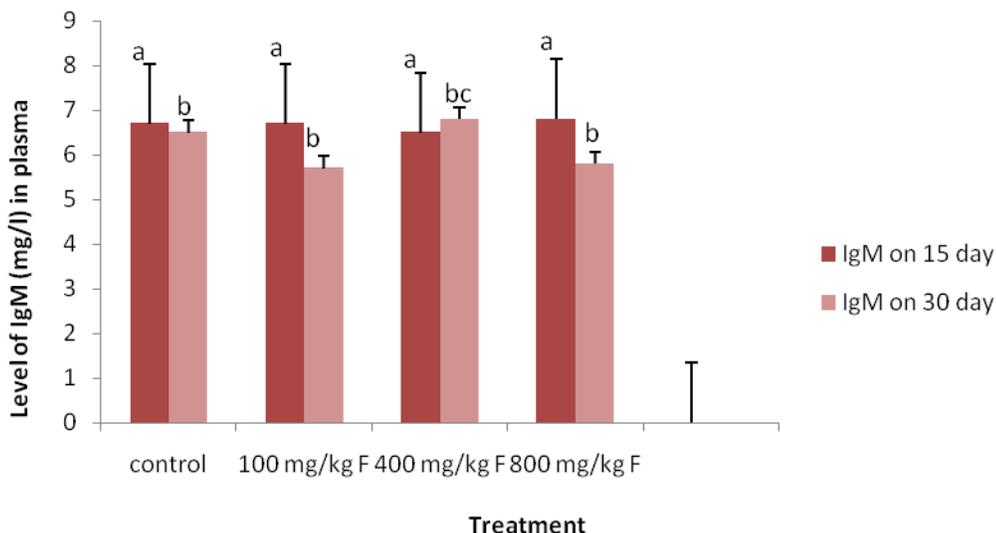


Fig. 1. Changes in immunoglobulin level in fish treated by diet containing the leaves and twigs extract of *Vitisvinifera*

Changes in level of immunoglobulin (IgM) in plasma of fish fed by a diet supplemented are not significant compared with the control group ($p < 0.05$).

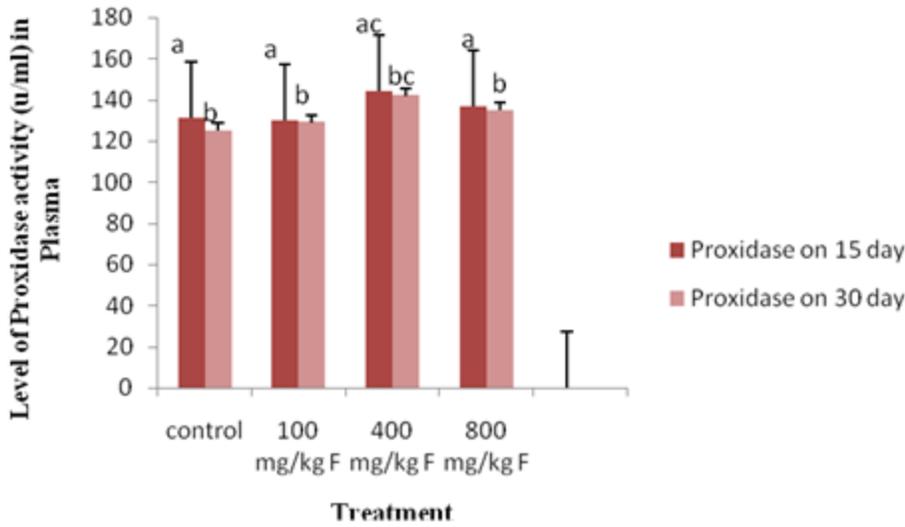


Fig. 2. Changes in the level of peroxidase activity in fish treated by diet containing the leaves and twigs extract of *Vitisvinifera*

Changes in peroxidase activity level in plasma of fish fed by treated by diet containing the leaves and twigs extract of *Vitisvinifera* (400 mg of leaves and twigs extract of *Vitisvinifera* per one kg of food) were significant compared with the control group ($p < 0.05$).

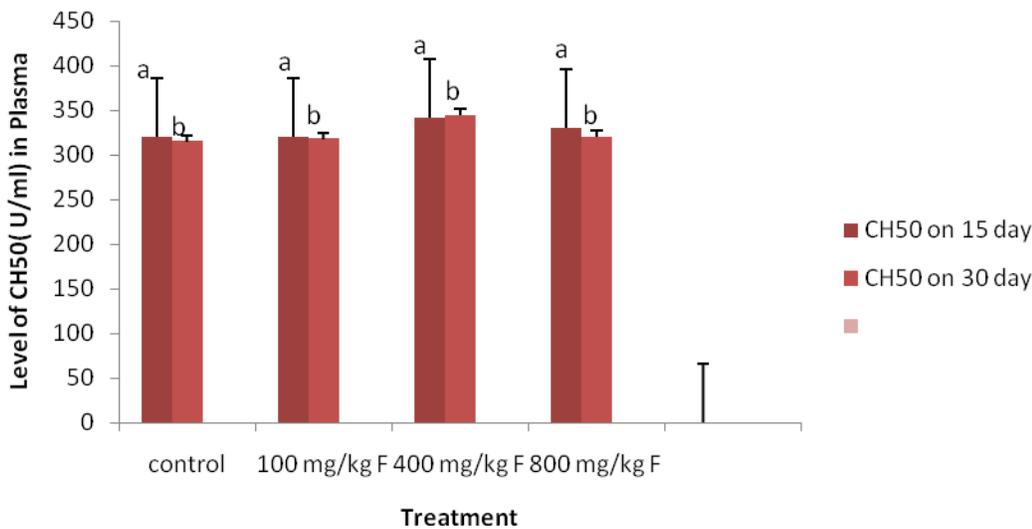


Fig. 3. Changes in the level of total complement in fish treated by diet containing the leaves and twigs extract of *Vitisvinifera*

Total complement level in plasma of fish fed by treated by diet containing the leaves and twigs extract of *Vitisvinifera* (400 mg of leaves and twigs extract of *Vitisvinifera* per one kg of food) was not significant compared with other experimental groups ($p < 0.05$).

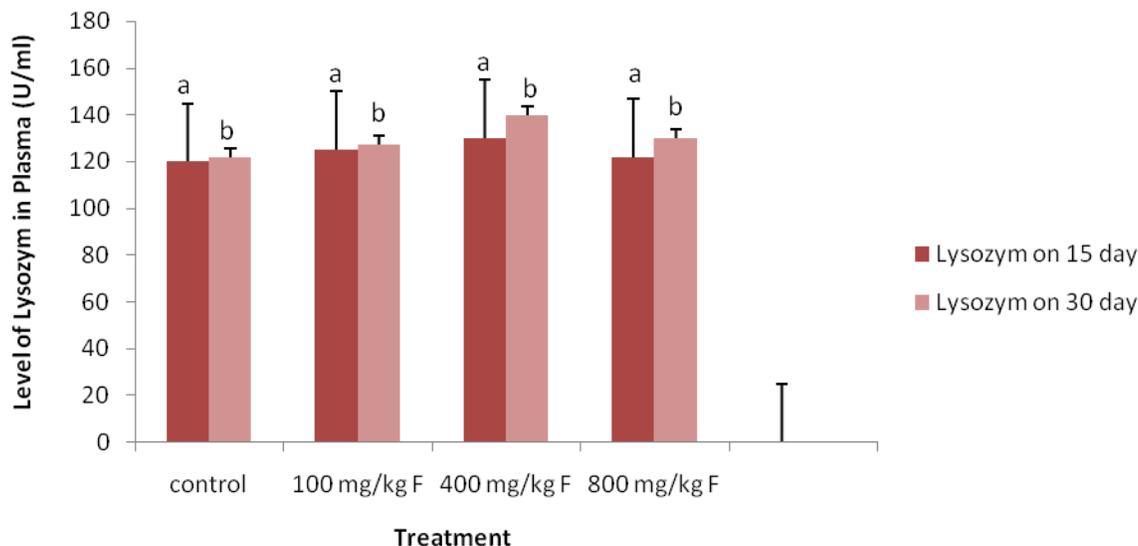


Fig. 4. Changes in the activity level of lysozyme in fish treated by diet containing the leaves and twigs extract of *Vitisvinifera*

Changes in activity level of lysozyme in plasma of fish fed by treated by diet containing the leaves and twigs extract of *Vitisvinifera* (400 mg of leaves and twigs extract of *Vitisvinifera* per one kg of food) were not significant ($p < 0.05$).

DISCUSSION

Maintaining the health and enhancing the immune system of fish against pathogens is one of the most important factors to increase their production at a macro level. Therefore, many fisheries researchers have conducted studies to achieve this important goal in recent decades. One method to increase the immune system of fish is to use of compounds stimulating the immune system called as immune-stimulants. These compounds include different types of chemical synthetic materials, and probiotics is a natural compounds having plant origin. By inhibiting 5-lipoxygenase and production of leukotrienes and free radicals of kupffer cells in rat liver, silymarin extract reduces inflammation of the liver [9] and prevents from the occurrence of cell damage and hemolysis of red blood cells in laboratory animals that have experimental poisoning [9, 10].

Medicinal use of silymarin can prevent inflammation of the brain cells and damage to central nervous system in laboratory animals [9]. The leaves and twigs of the *Vitisvinifera* plant can also inhibit the release of Milo peroxidase. This herbal medicine has anti-cancer property [10]. A significant increase was not observed ($p < 0.05$) in the IgM immunoglobulin level rainbow trout fish of *O. mykiss* fed by leaves and twigs of *Vitisvinifera* plant compared with control group was. David showed that the adding a mixture of sunflower seeds and vitamin in the diet of *Channa striata* fish increases the antibody and resistance of this fish against *Aphanomyces invadans*. Adding a dietary supplement containing extracts of *Catharanthus roseus* to Indian carp of *Labeo rohita* increased immune response of this fish [10]. Lysozyme activity level in fish fed by leaves and twigs of *Vitisvinifera* plant was relatively increased, but this increase was not statistically significant compared to the control group ($p < 0.05$). A significant increase in lysozyme activity level in a common carp of *Cyprinus carpio* fed by a mixture of traditional Chinese herbal medicines has also been reported [11]. Peroxidase activity level in experimental treatments fish, especially in fish fed by leaves and twigs of *Vitisvinifera* plant (400 mg per kilogram of leaves and twigs of *Vitisvinifera* plant), was significantly increased ($p < 0.05$). In addition, despite the relative increase in the total complement level in this fish, no difference was found in this fish compared with the control group ($p < 0.05$). The strengthening of immune system of Japanese yellow tail fish fed by nutritional supplements containing extracts of *Quillaja saponin* plant also demonstrated it [12]. The use of extracts of medicinal plants of *Rheum officinale*, *Andrographis paniculata*, *Lonicera japonica*, *Isatis indigotica* in diet of carp enhanced immune system of them [11]. Using turmeric powder and garlic in the diet of Indian larvae carp of *Catla catla* increased their resistance to disease and thus increased their survival rates in the rearing the fish period [13]. Based on the results, it can be

said that use of extracts of medicinal plants and vaccination of fish simultaneously can increase their specific immune against many pathogenic bacterial factors, including the *A. hydrophila* [12].

Despite the lack of a significant increase in immunoglobulin level in plasma of fish treated by leaves and twigs of the *Vitisvinifera* plant compared with control group fish, and an increase in peroxidase activity level in experimental treatment fish, especially in fish fed by food supplement of leaves and twigs of *Vitisvinifera* (400 mg per kilogram of leaves and twigs of *Vitisvinifera* plant), and the relative increase in the level of total complement and lysozyme activity in this species compared with the control group fish, it can be concluded that the leaves and twigs of the *Vitisvinifera* can have a positive impact on the immune system of fish.

CONFLICT OF INTEREST

Author declares no conflict of interest.

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