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Original Research

The effects of common yarrow (*Achillea millefolium* Linnaeus), cinnamon (*Cinnamomum zeylanicum* Blume) and rosemary (*Rosemarinus officinalis* Linnaeus) hydrosols on the some immunological and hematological parameters of common carp (*Cyprinus carpio* L., 1758) against to *Yersinia ruckeri*

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Abstract: In this study, the effects of some plant hydrosols (distilled plant waters) based upon some hematological parameters and Nitroblue Tetrazolium (NBT) activities in the common carp (*Cyprinus carpio* Linnaeus, 1758) infected with *Yersinia ruckeri* were investigated. In the trial, it was utilized totally 200 common carps with 54.3±6.7 g mean live weight and 15.7±1.8 cm mean total lenght. The 10% rate of the common yarrow (*Achillea millefolium* Linnaeus) hydrosol; 0.5% rate of the cinnamon (*Cinnamomum zeylanicum* Blume) hydrosol; and 5% rate of the rosemary (*Rosemarinus officinalis* Linnaeus) hydrosol were applied to fish as a bath treatment. The erythrocyte (RBC), leukocyte count (WBC), hematocrit value (HCT), haemoglobin amount (Hg), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and activities of NBT in the blood samples taken from the caudal vena of the control and experimental fish groups were analyzed in the 7th, 14th, and 21st days of the exposure treatment. At the end of the research, HCT, Hg, RBC, WBC, MCH and MCV values decreased in the C-2 Group (the control group contain pathogen) compared to the C-1 Group (the control group no contain pathogen), except MCHC value. The NBT activities in the C-1 Groups increased at the 14th day, but decreased quite a few at the 21st day. It has been consequently reached the conclusion that the bath treatments of the some plant hydrosols might be beneficial in increasing of antibacterial properties and in strengthening of defense mechanisms of common carp against *Y. ruckeri* pathogen.

Key words: Common Carp (*Cyprinus carpio*); *Yersinia ruckeri*; Hydrosol; Cinnamon, Rosemary; Common Yarrow.

Introduction

The treatment with herbal drugs, applied with the name of alternative medicines in humans, is also used in the ameliorating of the animal diseases, in the present. Currently, rised consumer demand for perfection in fish and shellfish farms, has put new dimensions to the quality, safety, elimination of concomitant pollutants, antibiotics, and carcinogens during the production process (1). In aquaculture, the plants or herbal sources were also used to improve the growth of the fish biomass (2); to effect the meat quality and body composition parameters (2, 3) and to improve sensory, chemical and microbiological properties of fish meat and fish products (3, 4), in addition to this, one of the most promising methods of controlling diseases is to strengthen nonspecific defense mechanisms in fish (5). In this context, plants or their by products are preferred since they contain several phenolic, polyphenolic, alkaloid, quinone, terpenoid, lectine, and polypeptide compounds many of which have been shown to be very effective alternatives to antibiotics, chemicals, vaccines, and other synthetic compounds (1). In the aquaculture systems, the herbal medicines are also known to exhibit anti-microbial activity, facilitate growth, and maturation of cultured species; besides under intensive farming the anti-stress characteristics of herbs will be of immense use with out posing any environmental hazard (6). In the previous studies, usage of the raw or powdered varieties of the different plants for adding into the culture water or feeds of the fish groups, has been researched (1, 7-9). It has emerged that hydrosol terapy (treatment with plant distilled water) has not been applied as well on the fish diseases.

The common carp as experimental live materials, is a more resistance fish species to the external effects and has commercial value, and however, is a preferred species in the experiments due to be a culturable fish. It was stated that the vacinations and the semptimatical treatments have not a permanent solution in the *Yersinia ruckeri* disease, caused the economical losses in the all of the other freshwater fish species, mainly carps and trouts (10). The chemotherapeutics that do not prevent the latent course of the disease, can not prevent the deasease and, cause to raise the resistance of the pathogen to used drugs. However, the fish is open to other pathogens being in the environment depend on their effects.

Some studies on the fish hematology have reported that some blood parameters are helpful in the diagnosing of the most diseases. The erythrocytes (RBC) and leukocytes (WBC) counts, and haematocrit (HCT), haemoglobin (Hg) and total plasma protein (TPP) val-

ues decreased in the rainbow trouts infected with the *Y. ruckeri* (11).

The common yarrow (*Achillea millefolium* Linnaeus) is a plant containing volatile/essential oils including azulene, limonene, sineol, borneol, pinene, and sesquiterpenes. However, it is also a plant used because of anti-inflammatory, neuroleptic, antiviral, contraceptive, diuretic menstrual cramps, intestinal irritation, stimulant, and antifungal effects. Its antibacterial effects are well enough, even when it given at low doses in the fish feed ration (12-15). The cinnamaldehyde in volatile oil in cortex cinnamomi has the antibacterial, fungistatic and raising the motility features. There are so many medical usage of the cinnamon. It has been reported that even the low doses of Cinnamon, like the same yarrow, have a quite strong antibacterial effect (16). The antibacterial, antioxidant, antiviral, and immune system improving effects of rosemary have been previously revealed (17, 18). The antibacterial substances in the rosemary especially play an active role in the structural deformation of morphological structure of the bacterium, and the cell wall and cytoplasm (19).

Because of the difficulties and high cost of obtaining the plant extracts, the researchers have been referred to a practical and effective method of both of usage and production, such as plant hydrosols. The lack of researches about the plant by-products like hydrosols on fish, until now, has been seen. Especially, both hydrosols contain water-soluble substances can be easily absorbed by the fish depending on its ability due to dissolve in water, and the lethal doses of the used hydrosols had been previously determined, were the most effective approach of the present study. In this study, it was aimed to determinate that the hydrosols cause what kind of changes in the fish against to bacterium; and application fields of the hydrosols, and to be helpful for improving the new methods, with the following the changes depending on the durations in the blood parameters of the fish, after the experimental applications. For the aquaculture, the effects of one of the most effective pathogens on the fish losses in the fish culture systems, might be determined and a new approach could be used in its treathment.

Materials and Methods

Material

Experimental Materials

This study was conducted in the Research and Application Unit of Fisheries Faculty of İnönü University (Malatya, Turkey). In the experiments, it was utilised totally 5 fiberglass culture tanks (250 l), disinfected and filled with 150 l water stable throughout the trial. The tanks were continously aerated. The mean water quality parameters values in the trials were generally as follow: dissolved oxigen; 6.4 mg l^{-1}, water tempeature; 17.1 °C, pH; 7.37, conductivity; 500.4 µS, salinity; 225.4 g l^{-1}, and total dissolved materials; 345.6 mg l^{-1}.

The common carp (*Cyprinus carpio* L., 1758) was supplied from Keban Aquaculture Branch Office of Directorate General for State Hydraulic Works (DSİ), Ministry of Forestry and Water Affairs (Turkey). The common carps were transported to laboratory and acclimizated to the laboratorial conditions for two weeks and sick fish was observed and removed from the trial. Totally, 200 common carps (54.3±6.7 g and 15.7±1.8 cm) and 40 for every experimental group were used in the study. Every fish individual was used as a replicate. The fish were fed with the standard commercial carp feeds as at libitum.

The hydrosols were supplied from a commercial company (Kırkambar Spice Company, Turkey). The hydrosols of each plant were obtained for 1 h in a hydrodistillation apparatus with 500 ml water (1-Plant-: 10-Water-; w/v). Then, the oil was removed by separation funnel. The hydrosols were kept in sterile dark colored bottles (500 ml) under refrigerated conditions until use (20, 21).

The *Yersinia ruckeri,* applied to fish after the acclimization periods, contains $1.5x10^8$cfu ml^{-1} calculated by 0.5 McFarland opasite methods (22) and obtained from YR11 lyophilized pure yersina strain, provided from the Eğirdir Fisheries Faculty, Süleyman Demirel University (Isparta, Turkey). Before usage in experimental infections, bacteria were sub cultured on triptych soy agar (TSA, Oxoid) to check purity, and then cultured in triptych soy broth (TSB).

Experimental Design

The research groups were designed as control groups (C-1 and C-2) and experimental groups (D-1, D-2, and D-3). C-1: the control group contains 0.0% hydrosol and no pathogen; C-2: the control group contains 0.0% hydrosol and pathogen; D-1: the group contain 10.0% common yarrow hydrosol and pathogen; D-2: the group contain 0.5% cinnamon hydrosol and pathogen; and D-3: the group contain 5.0% rosemary hydrosol and pathogen.

The *Yersinia ruckeri* inoculum (0.1 ml) was intraperitoneally applied to infected fish groups (C-2, D-1, D-2, and D-3), after the acclimization periods. At the same time, the PBS (Phosphate buffered saline) was also intraperitoneally applied to control fish group (C-1). After the 24 hours of the injection, all of the fish was exposed to hydrosols as bath treatment. In this application, the fish were kept in the fish tanks contained the different hydrosols for a half hour in a day during the 3 days. In the 7^{th}, 14^{th}, and 21^{st} days of the application (durations), the blood samples of fish (n=10) from all groups were taken and analysed in the same day. The animal experiments in this study were performed in accordance with the rules in guidelines for animal research and health from the National Institute of Health and with the approval of Ethics Board of Experimental Animals in Inönü University (Malatya, Turkey).

Methods

LC_{50} Determination

A pre-experiment was conducted to determinate the LC_{50} values of the used hydrosols (common yarrow, cinnamon and rosemary). In this experiment, 10.00, 20.00, 22.50, and 30.00 rates (%) of common yarrow determined by a pre-research; 0.50, 1.00, 2.50, 5.00, and 10.00 rates (%) of cinnamon (23); and 5.00, 7.50, and 10.00 rates (%) of rosemary (24) were applied to the common carp.

Analysis of Hematologic Parameters

In the 7th, 14th, and 21st days of the application, the blood samples of fish (n=10) randomly selected from all groups were taken. Fish were anesthetized with the 0.30 ml L^{-1} 2-phenoxyethanol for 10 minutes (25) and observed anesthesia of fish under deep sedation, including loss of swimming action and partial loss of equilibrium (26). The blood samples were taken from the caudal vena into K2 EDTA tubes (4 ml). The HCT, Hg, RBC, WBC, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), and the activity of NitroblueTetrazolium (NBT) in the blood samples and analysed by fully automatic hematology analyser (PROCAN PE-6800VET Brand) in the same day.

Analysis of NitroblueTetrazolium (NBT) Activity

The 0.1 ml of 0.2 % NBT solution was added to the microtiter plates and an equal amount of blood was added on it. After this suspension was allowed to stand at room temperature for 30 minutes, 0.05 ml of it was added to glass tubes containing 1 ml of N, N-dimethylaformamide. After centrifugation at 3000 rpm for 5 minutes (centrifuge; Nuve Brand NF800 Model), the upper layer was read on a spectrophotometer (Hach Lange Brand DR6000 Model) at 540 nm.

Statistical Analysis

One-way ANOVA and the Duncan Multiple Range Test were used to determine the statistical differences in parameters among all of the experimental groups and the durations ($P<0.05$). The mean values were given in the results as mean ± standart error of means. However, all results were analysed by the SPSS 24.0 Package Programme.

Results

LC_{50} Experiment

In the LC_{50} experiment, 10.00 % rate of common yarrow, 0.50 % rate of cinnamon and 5.00 % rate of rosemary were determined as LC_{50} concentrations for the common carps.

Hematological Parameters

All of the results of mean values of the hematological parameters in the common carps exposed to different hydrosols depending on the different durations, was given in the Table 1, 2, 3, and 4.

The HCT values decreased in the C-2 (the control group contain pathogen) compared to the C-1 (the control group no contain pathogen). The HCT values increased in the all of the experimental groups (D-1; Common yarrow Hydrosol, D-2; Cinnamon Hydrosol, and D-3; Rosemary Hydrosol) and all experimental durations (7th, 14th, and 21st days of the application). The HCT values of the C-1 and C-2 Groups showed generally an increase in the durations ($P<0.05$), while there is a significant increase in only D-3 Group ($P<0.05$). The HCT values of the all groups were statistically found different in the durations ($P<0.05$) except in the 14th day ($P>0.05$) (Table 1.).

Table 1. Hematocrit value (HCT, %) and haemoglobin amount (Hg, g/dL) of common carp (*Cyprinus carpio* L., 1758) exposed to some hydrosols applications against *Yersinia ruckeri.*

Groups		Parameters					
		Hct			Hg		
		7th Day	14th Day	21st Day	7th Day	14th Day	21st Day
Control Groups	C-1	$33.44\pm2.03_{a}^{AB}$	$32.30\pm1.27_{a}^{A}$	$44.70\pm0.06_{b}^{B}$	$15.02\pm0.35_{b}^{A}$	$13.50\pm0.10_{a}^{A}$	$15.50\pm0.09_{b}^{B}$
	C-2	$30.32\pm1.75_{a}^{A}$	$30.45\pm0.63_{a}^{A}$	$35.83\pm2.08_{b}^{A}$	$13.82\pm0.54_{a}^{A}$	$13.88\pm0.42_{a}^{A}$	$13.23\pm0.81_{a}^{A}$
Experimental Groups	D-1	$40.78\pm3.23_{a}^{B}$	$39.53\pm8.09_{a}^{A}$	$39.90\pm3.55_{a}^{AB}$	$17.62\pm1.73_{a}^{A}$	$17.27\pm3.27_{a}^{A}$	$14.50\pm0.80_{a}^{AB}$
	D-2	$38.66\pm4.31_{a}^{AB}$	$36.50\pm4.42_{a}^{A}$	$39.40\pm0.70_{a}^{AB}$	$16.94\pm2.33_{a}^{A}$	$16.20\pm2.01_{a}^{A}$	$13.55\pm0.43_{a}^{AB}$
	D-3	$35.14\pm1.18_{a}^{AB}$	$36.95\pm2.40_{b}^{A}$	$41.32\pm1.80_{b}^{AB}$	$14.22\pm0.37_{a}^{A}$	$15.68\pm0.34_{b}^{A}$	$14.50\pm0.51_{ab}^{AB}$

Different letters (A and B) show statistical differences of "Duncan Multiple Range Test" among all groups (controls and experimental groups) in same exposure durations (7th, 14th and 21st day) for same parameter and column. Different letters (a and b) show statistical differences of "Duncan Multiple Range Test" among exposure durations (7th, 14th and 21st day) in same group (controls and experimental groups) and line.

Table 2. The erythrocyte (RBC, $10^6/\mu L$) and leukocyte (WBC, $10^3/\mu L$) counts of common carp (*Cyprinus carpio* L., 1758) exposed to some hydrosols applications against *Yersinia ruckeri.*

Groups		Parameters					
		RBC			WBC		
		7th Day	14th Day	21st Day	7th Day	14th Day	21st Day
Control Groups	C-1	$2.00\pm0.12_{a}^{A}$	$1.77\pm0.02_{a}^{A}$	$2.11\pm0.01_{a}^{B}$	$47.94\pm1.33_{a}^{AB}$	$50.97\pm1.56_{a}^{A}$	$72.90\pm0.23_{b}^{A}$
	C-2	$1.87\pm0.05_{a}^{A}$	$1.86\pm0.04_{a}^{A}$	$1.73\pm0.13_{a}^{A}$	$41.84\pm2.39_{a}^{A}$	$50.33\pm3.01_{ab}^{A}$	$63.03\pm8.02_{b}^{A}$
Experimental Groups	D-1	$2.34\pm0.23_{a}^{A}$	$2.26\pm0.44_{a}^{A}$	$1.94\pm0.18_{a}^{AB}$	$59.90\pm6.10_{a}^{B}$	$59.20\pm13.96_{a}^{A}$	$64.60\pm5.97_{a}^{A}$
	D-2	$2.33\pm0.33_{a}^{A}$	$2.13\pm0.27_{a}^{A}$	$1.77\pm0.08_{a}^{AB}$	$57.90\pm7.32_{a}^{B}$	$55.60\pm9.10_{a}^{A}$	$58.70\pm4.91_{a}^{A}$
	D-3	$1.96\pm0.09_{a}^{A}$	$2.03\pm0.03_{a}^{A}$	$1.97\pm0.09_{a}^{AB}$	$51.86\pm2.57_{a}^{AB}$	$58.22\pm2.36_{a}^{A}$	$57.80\pm4.09_{a}^{A}$

Different letters (A and B) show statistical differences of "Duncan Multiple Range Test" among all groups (controls and experimental groups) in same exposure durations (7th, 14th and 21st day) for same parameter and column. Different letters (a and b) show statistical differences of "Duncan Multiple Range Test" among exposure durations (7th, 14th and 21st day) in same group (controls and experimental groups) and line.

The Hg values decreased slightly in C-2 Group compared to C-1 Group in the every duration. The application of the hydrosols increased the Hg values. The differences among the groups are statistically significant in only 21st day ($P<0.05$), it was found different in the only C-1 and D-3 Groups among the durations ($P<0.05$) (Table 1.).

In the C-1 Group, the RBC decreased in the 14th day and then, increased in the 21st day. While the WBC values increased in the durations. All the RBC and WBC values decreased in the C-2 with pathogenic effect, and increased in the all of the hydrosol applications. In only 21st day, there are a statistical significance in the RBC values among the groups ($P<0.05$) and in the WBC values in the 7th day ($P<0.05$). There are only statistical differences in only C-1 and C-2 Groups in the durations ($P<0.05$) (Table 2.).

With the pathogenic effect, the MCH values decreased, but MCHC values increased in the C-2 Group compared to C-1 Group. It was found statistically differents in the MCH values of D-2 Group and the MCHC values of all groups among the durations ($P<0.05$), and in the MCHC values in the 7th and 14th days among groups ($P<0.05$) (Table 3.).

The MCV values decreased in C-2 Group compared to C-1 Group in the every duration and the application of the hydrosols increased it. However, the values increased in the durations. The all of differences among the groups and durations ($P<0.05$) except 21st day ($P>0.05$) is statistically significant (Table 4.).

NBT Activity

The NBT activities in the C-1 Groups increased in the 14th day, but decreased quite a few in the 21st day. In the C-2 Groups with the pathogenic effect and D-1 Group, the NBT activities generaly increased in the all durations, but in the 14th day, it increased and then, decreased in the 21st day. The NBT activities decreased in the durations in case of D-2 and D-3 Groups. The statistically significant differences were found in the all times among the groups and in the all groups among the durations ($P<0.05$), except C-2 Group ($P>0.05$) (Table 4.).

Discussion

In this study, it was aimed to determinate that the hydrosols cause what kind of changes against to bacterium; and application fields of the hydrosols, and to be helpful for improving the new methods, with the following the changes depending on the durations in the blood parameters, after the experimental applications. Antibacterial properties of plant hydrosols against to *Y. ruckeri* were determined by changes of some blood parameters and NBT activity. Hematological parameters are frequently used in assessment of disease and applications of drug-treatment (27). Some studies on the fish hematology have reported that some blood parameters are helpful in the diagnosing of the most diseases. The RBC and WBC counts, and HCT, Hg and total plasma protein (TPP) values decreased in the rainbow trouts infected with the *Y. ruckeri* (11). With the pathogenic effect, all of the blood parameters (HCT, Hg, RBC, WBC,

Table 3. The mean corpuscular haemoglobin (MCH, pg) and mean corpuscular haemoglobin concentration (MCHC, g/dL) values of common carp (*Cyprinus carpio* L., 1758) exposed to some hydrosols applications against *Yersinia ruckeri.*

Groups		Parameters					
		MCH			MCHC		
		7th Day	14th Day	21st Day	7th Day	14th Day	21st Day
Control Groups	C-1	$75.82\pm2.86_{a}^{A}$	$76.07\pm0.38_{a}^{A}$	$73.40\pm0.12_{a}^{A}$	$45.32\pm1.98_{b}^{B}$	$41.87\pm1.78_{b}^{AB}$	$34.60\pm0.17_{a}^{A}$
	C-2	$73.72\pm1.59_{a}^{A}$	$74.45\pm1.17_{a}^{A}$	$76.75\pm1.49_{a}^{A}$	$45.76\pm0.97_{b}^{B}$	$45.50\pm0.89_{b}^{C}$	$36.88\pm0.47_{a}^{A}$
Experimental Groups	D-1	$75.40\pm0.96_{a}^{A}$	$76.40\pm1.07_{a}^{A}$	$75.40\pm3.11_{a}^{A}$	$42.94\pm1.08_{b}^{AB}$	$43.90\pm0.87_{b}^{BC}$	$36.57\pm1.37_{a}^{A}$
	D-2	$72.80\pm0.68_{a}^{A}$	$76.25\pm0.48_{b}^{A}$	$76.85\pm0.95_{b}^{A}$	$43.42\pm1.35_{b}^{AB}$	$44.30\pm0.62_{b}^{BC}$	$36.85\pm1.24_{a}^{A}$
	D-3	$73.02\pm2.04_{a}^{A}$	$77.34\pm2.21_{a}^{A}$	$73.62\pm0.82_{a}^{A}$	$40.48\pm0.77_{b}^{A}$	$39.74\pm0.27_{b}^{A}$	$35.12\pm0.51_{a}^{A}$

Different letters (A, B, and C) show statistical differences of "Duncan Multiple Range Test" among all groups (controls and experimental groups) in same exposure durations (7th, 14th and 21st day) for same parameter and column. Different letters (a and b) show statistical differences of "Duncan Multiple Range Test" among exposure durations (7th, 14th and 21st day) in same group (controls and experimental groups) and line.

Table 4. The mean corpuscular volume (MCV, fL) value and activity of Nitroblue Tetrazolium (NBT, mg/ml) of common carp (*Cyprinus carpio* L., 1758) exposed to some hydrosols applications against *Yersinia ruckeri.*

Groups		Parameters					
		MCV			NBT		
		7th Day	14th Day	21st Day	7th Day	14th Day	21st Day
Control Groups	C-1	$167.84\pm3.62_{a}^{AB}$	$182.43\pm7.52_{a}^{BC}$	$212.20\pm0.12_{b}^{A}$	$0.188\pm0.010_{b}^{A}$	$0.192\pm0.019_{b}^{A}$	$0.068\pm0.009_{a}^{A}$
	C-2	$161.84\pm6.15_{a}^{A}$	$164.00\pm2.72_{a}^{A}$	$208.43\pm6.35_{b}^{A}$	$0.192\pm0.005_{a}^{A}$	$0.224\pm0.019_{a}^{AB}$	$0.181\pm0.014_{a}^{B}$
Experimental Groups	D-1	$176.16\pm5.53_{a}^{AB}$	$174.43\pm5.09_{a}^{AB}$	$206.57\pm1.75_{b}^{A}$	$0.202\pm0.006_{a}^{A}$	$0.256\pm0.015_{b}^{B}$	$0.196\pm0.013_{a}^{BC}$
	D-2	$168.44\pm5.30_{a}^{AB}$	$172.25\pm1.65_{a}^{AB}$	$209.25\pm4.36_{b}^{A}$	$0.420\pm0.002_{b}^{C}$	$0.260\pm0.019_{a}^{B}$	$0.260\pm0.031_{a}^{BC}$
	D-3	$180.50\pm4.07_{a}^{B}$	$194.60\pm5.00_{b}^{C}$	$209.96\pm2.49_{b}^{A}$	$0.391\pm0.015_{c}^{B}$	$0.330\pm0.009_{b}^{C}$	$0.245\pm0.017_{a}^{C}$

Different letters (A, B, and C) show statistical differences of "Duncan Multiple Range Test" among all groups (controls and experimental groups) in same exposure durations (7th, 14th and 21st day) for same parameter and column. Different letters (a, b, and c) show statistical differences of "Duncan Multiple Range Test" among exposure durations (7th, 14th and 21st day) in same group (controls and experimental groups) and line.

and MCV) conformably decreased, but MCHC values and NBT activities increased in the C-2 Group compared to C-1 Group. In a different study, evaluated the antibacterial effects of gum tree on the common carps, it was states that the HTC, Hg, RBC, WBC, MCV and MCHC values decreased in the Control Group and in the experimental groups, all parameters except MCHC, increased compared to infected group as similar to present results (C-2 Group in the present study) (28). *Y. ruckeri* infection decreased the HCT, Hg, RBC, WBC and MCV values in the common carp according to Control Group, while MCHC value decreased (29).

In a research, the extracts of the *Rheum officinale, Andrographis paniculata, Isatis indigotica* and *Lonicera japonica* plants were applied and determined an increase in the WBC count in the groups applied the extracts (30). The increase in the WBC count in the experimental groups (D-1, D-2, and D-3) compared to infected group (C-2) by the *Y. ruckeri,* it was evaluated as an indication of the increasing of phagocytosis, and immunostimulant effects.

Baba et al. (31) examined the effects of oat, *Avena sativa* extract on the non-specific immune system of common carp (*Cyprinus carpio*), injected with *Aeromonas hydrophila.* Hg, MCH, and MCHC values increased in the experimental group fed with the supplemented diet with oat extract, paralelly the present results. They stated that their results suggest that *A. sativa* extract like the hydrosols of common yarrow, cinnamon and rosemary, can be used as a feed supplement to enhance fish immune response and disease resistance against *A. hydrophila.*

Harikrishnan and Balasundaram (32) evaluated the antimicrobial potency of aqueous and ethanolic decoction (individual extract) and concoction (mixed extract) of three common medicinal herbs; turmeric, *Curcuma longa*, tulsiplant, *Ocimum sanctum*, and neem, *Azadirachta indica*, against the in vitro growth of *Aeromonas hydrophila* in the goldfish, *Carassius auratus.* They had reported in this study that *A. indica* exhibited the most potent antibacterial property ($P< 0.05$) against *A. hydrophila* among the decoctions and among the concoctions, both the aqueous and ethanolic triherbal extracts mixed in the ratio of 1:1:1 had higher antibacterial activity ($P<0.05$) than the other concoctions and decoctions. The RBC count, Hg and HCT values of the infected group were significantly higher ($P<0.05$) than those of the control group. In the early treated group, all of the affected profile values returned to near normal, while the late-treated group registered a partial recovery, such as improved RBC count. The derived hematological values, such as MCV, MCH, and MCHC, of the early and late-treated groups also significantly declined ($P<0.05$), but were restored to near normal ($P<0.05$) only in the early treated group. They emphasized that the results of their research suggest that dip treatment of *A. hydrophila*-infected goldfish in an aqueous triherbal concoction had a synergistic restorative effect on the hematological variables. Besides, it was informed that are storative increasing of RBC count in the fish species firstly infected by bacteria and secondly applied a herbal decoctions or concoctions, has strengthened the natural immune system of the fish (33).

The decrease in the RBC count and Hg levels in the C-2 Group, shows a hypochromic microcytic anaemia caused by *Y. ruckeri*. The increase in the RBC count and Hg levels in the experimental groups shows also, that both of the pathogen caused the stress and the anaemia sitimulate the immune system of the fish to activate the negative feedback mechanism of the fish (34).

Sharma et al. (35), reported that the NBT activities which is a non-specific immune response indicator, of roats of the *Withania somnifera* plant in the *Labeo rohita* against to *A. hydrophila,* showed an increase similarly to results of the present study. At the same time, the high activities of NBT, which is an indicator of the the oxygen radical production capacity of the neutrophils, in the especially cinnamon and romemary hydrosol groups (D-2 and D-3, respectively) revealed the strengthened phagocytosis.

With the pathogenic effect, all of the blood parameters (HCT, Hg, RBC, WBC, and MCV) conformably decreased in the C-2 Groups, except MCHC values and NBT activities compared to C-1 Group depending on the infection. However, it was observed that in the all of the experimental groups, all parameters except MCHC, increased and shown a recovery to values of C-1 Group compared to infected group (C-2). When the compare the antibacterial effects of the three plant hydrosols against to *Y. ruckeri*, in general terms, it was evaluated that the most effective hydrosol is common yarrow hydrosol (D-1) followed by cinnamon (D-2) and rosemary (D-3) hydrosols, respectively.

In a conclusion, it has been reached that the bath treatments of the three plant hydrosols might be helpful for the recovery of both the hematopoetic and immune systems

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Authors' Contributions

All responsibilities and contributions of every author are equal.

Conflict of Interest Disclosure

There is no conflict of interest among authors of this manuscript.

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