# Comparison of the effects of propolis and pollen extracts in the same concentrations on some biochemical and hematological parameters in rainbow trout (*Oncorhynchus mykiss*)

**Fuat Gulhan M.[1*]; Selamoglu Z.[2]**

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**Abstract**

Three different concentrations (10, 20 and 30 ppm) of the dry propolis and pollen extracts were applied in double distilled water. Biochemical and hematological parameters in blood of rainbow trout (*Oncorhynchus mykiss*) treated to various propolis and pollen concentrations (10, 20 and 30 ppm) for 96 h were determined. Fish treated to 20 and 30 ppm of propolis extract presented significant increases ($p$<0.05) in glucose, triglycerides, total cholesterol levels and LDH activities as compared with the 10 ppm. But, there were significant decreases in AST, ALP, erytrocyte and hemoglobin as compared with the 10 ppm. In groups administrated pollen extracts; there were significant decreases in the levels of urea and glucose in 10 and 20 ppm concentrations compared with 30 ppm ($p$<0.05). Significant changes in the erytrocyte counts and hematocrit values in blood of fish treated to 20 and 30 ppm pollen were observed compared to 10 ppm pollen group ($p$<0.05). The data showed that propolis and pollen have important positive effects on biochemical and hematological parameters at 10 ppm levels, whereas other concentrations appear to be unfavorable for blood of rainbow trouts. Due to antioxidant and preservative properties of propolis and pollen, they may not only prolong the physiological functions of some aquatic living organisms, but also contribute to the health benefit of consumers who consume aquatic animals.

**Keywords:** Biochemical parameter, Hematological parameter, Pollen, Propolis, Rainbow trout

1-Department of Medicinal and Aromatic Plants, Technical Sciences Vocational School, Aksaray University, 68100 Turkey
2-Department of Biotechnology, Faculty of Arts and Science, Niğde University, 51240 Turkey
***** Corresponding author's email: mfuatgulhan@aksaray.edu.tr



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## Introduction

Fish and fishery products have been identified as nutritional sources because of their high protein content. However, their biochemical changes which causes substantial problems in distribution (Morales-Medina *et al*., 2016). The limitation of natural resources such as fresh water and land has led to intensification of production systems. This overcrowding and the other stress conditions are likely to produce poor physiological environment and increase susceptibility to infectious diseases (Trenzado *et al*., 2008). Moreover, nutrition has an influence on health and immune responses of fish; therefore, research into dietary immunostimulant supplements such as organic, inorganic and synthetic matters has increased and many agents (such as various natural antioxidants) are currently used in the aquaculture industry (Sakai, 1999).

Nowadays, various natural products such as herbal extracts, microalgae and yeasts are known to have important roles in improving defense system due to their antioxidant, antistress, antimicrobial, growth promotion and immunostimulation activities. Moreover, these types of natural immunostimulants have been shown to have less negative side effects and more cost effective than the commercial ones (Harikrishnan *et al*., 2003; Supamattaya *et al*., 2005; Salnur *et al*., 2009). Among several natural antioxidants, propolis and pollen have received particular attention due to their in vitro and in vivo immunostimulation effects. The ethnopharmacological approach, combined with chemical and biological methods, may provide useful pharmacological leads. Several researchers have reported the antitumoral property of propolis both *in vivo* and *in vitro* (Banskota *et al*., 2001a,b; Lee *et al*., 2005). Propolis has been used since ancient times as a medicine because of its biological properties as antimicrobial, antifungal, antiprotozoan and antiviral agents (Alberto *et al*., 2005). Honeybee-collected pollen is an apicultural product which is composed of nutritionally valuable substances and contains considerable amounts of polyphenolic compounds, mainly flavonoids, which may act as potent antioxidants (Marghitas *et al*., 2009). Organisms have endogenous antioxidants as well as exogenous taken. Fish are commonly used to estimate the influences of environmental factors due to the sensitivity of their biochemical and hematological parameters under certain conditions (Lopes *et al*., 2001). As a sign of stress, the using of biochemical and hematological methods provides valuable knowledge about physiological reactions occured against to changing environmental conditions. Biochemical and hematological parameters of fish are determined as an index of their health status as well (Coşkun *et al*., 2016).

This study was aimed to comparison of the effective concentrations of these bee products on biochemical and



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hematological parameters in blood of rainbow trout.

## Materials and methods

*Animals and experimental design*

The rainbow trouts (*O. mykiss*) were obtained from Camardı, Ecemis fish farm (Nigde, Turkey). Fish were hold for 15 days in a 8 x 5 x 1.5m stock tank to be acclimatized. After environmental adaptation period, 49 rainbow trouts were distributed into seven groups, each consisting of seven animals. They were transfered to tank, which filled with 200 L well aerated water (physical and chemical parameters of water are shown in Table 1). Artificial dry food was provided for once daily. Fish, which are using in this study had an average weight of 240–250 g and and length of 29.35 ± 1.14 cm.

*Preparation of propolis and pollen extractive solution*

Propolis and pollen were dissolved to 30% in ethanol, protected from light and moderately shaken for 1 day at room temperature. Afterward, the extracts were filtered twice, dried and stored in sealed bottles at 4 $^0$C until use. Three different concentrations (10, 20 and 30 ppm) of the dry propolis and pollen extracts were achieved in double distilled water (Talas and Gulhan, 2009; Talas *et al*., 2012).

*Experimental design*

There were totally forty nine rainbow trouts in 10, 20 and 30 ppm propolis and pollen, respectively treated groups and control group. Forty nine rainbow trouts were divided into seven groups with control, which treated to 10 ppm pollen and propolis, 20 ppm pollen and propolis, 30 ppm pollen and propolis. Fish experiments were performed in accordance with the guidelines for approving by the Committee of Animal Experiments at Cumhuriyet University, Sivas, Turkey.

*Biochemical assay*

After application for 96 hours, fish were anaesthetised with 40 mg/L of clove oil (Mylonas *et al*., 2005) and 2 mL of blood was obtained from caudal vein. Blood samples were transferred to tubes, kept into cooled bath and immediately analysed. The blood was centrifuged at 3000 x g, at 4ºC for 5 minutes. All of the analyses were done with an Olympus Optical Corp. (Shizuoka-ken, Japan), using commercially available kits (Roche).

*Hematological analyses*

Blood samples were collected from caudal vein of fish. The blood samples were transferred to tubes. Red blood cell counting was done after 1:200 dilution into Hayem solution. Counting leucocytes was done in blood samples after dilution into Turck solution (Blaxhall, 1981). Hemoglobin (Hb) concentration was determined according to the cyano-methemoglobin procedure (Kit 525- A; Sigma Chemical, St. Louis, MO, USA) (Blaxhall and Daisley, 1973).



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**Table 1: Amount of physical and chemical parameters of water during the present study.**

| Parameters | Before treatment | After treatment |
|---|---|---|
| Dissolved oxygen (ppm) | 7.8 ± 0.2 | 7.6 ± 2.3 |
| Chemical oxygen demand (ppm) | 14.1 ± 0.6 | 16.5 ± 1.2 |
| Suspended solids (ppm) | 37.6 ± 1.5 | 42.1 ± 1.2 |
| Calcium (ppm) | 128.0 ± 0.8 | 109.1 ± 1.5 |
| Sodium (ppm) | 22.7 ± 0.4 | 18.1 ± 0.5 |
| Chloride (ppm) | 14.0±1.2 | 15.4±1.8 |
| Total nitrogen (ppm) | 5.6 ± 0.5 | 6.5 ± 0.7 |
| Hardness ($CaCO_3$) (ppm) | 169.3 ± 2.6 | 161.4 ± 2.1 |
| Temperature ($^oC$) | 12.8 ± 1.6 | 11 ± 1.3 |
| pH | 7.6 ± 0.1 | 7.6 ± 0.1 |

Nonclotted blood (20 µL aliquots) was diluted with 1 mL of Drabkin solution and left to stand for 10 min at room temperature. The absorbance was read at 540 nm. The amount of Hb was calculated against to a Hb standard (Azizoglu and Cengizler, 1996). Hematocrit was determined according to Jewet et al. and Wilhelm Filho et al. (Jewet *et al.,* 1991; Wilhelm Filho *et al*., 1992). Nonclotted blood was transferred to a microhematocrit capillary, centrifuged at 14,000g for 5 min and read against to a standart cart.

*Statistical analysis*

Hematological and biochemical data were analyzed with SPSS 20.0 for Windows using one-way analyses of variance (ANOVA). Differences between means were determined using Duncan's multiple range test in which the significance level was defined as $p<0.05$.

**Results and Discussion**

We investigated the effective and useful concentrations of propolis and pollen, which have biologically benefits exists in natural environmental, on living organisms in natural area. It is well known that polyphenols are responsible for the antioxidative and radical scavenging activities of natural agents. In this respect, bee products are receiving a special attention in the field of functional foods and medicines. (Xu *et al*., 2009). This study is first research as comparison on effective concentration of propolis and pollen on biochemical and hematological parameters in blood of rainbow trout.

Changes in biochemical and hematological parameters in blood of rainbow trout caused by application of propolis and pollen extracts are presented in Tables 1 and 2. Fish treated to 20 and 30 ppm of propolis extracts presented significant increases ($p<0.05$) in glucose, triglycerides, total cholesterol levels and LDH activities as compared with the 10 ppm.



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**Table 2: Changes on the biochemical parameters in rainbow trout blood treated to 10, 20 and 30 ppm propolis and pollen concentrations.**

| Groups / Parameters | Control | Propolis 10 ppm | Pollen 10 ppm | Propolis 20 ppm | Pollen 20 ppm | Propolis 30 ppm | Pollen 30 ppm |
|---|---|---|---|---|---|---|---|
| **Metabolits** | | | | | | | |
| Glucose (mg $dL^{-1}$) | 76.5±0.5$^{b}$ | 66.1±0.6$^{c}$ | 72.5±0.8$^{b}$ | 74.2±0.8$^{b}$ | 74.0±1.7$^{b}$ | 80.3±1.1$^{a}$ | 75.5±2.1$^{b}$ |
| Total protein (g $dL^{-1}$) | 2.86±0.07$^{b}$ | 2.60±0.08$^{b}$ | 3.44±0.8$^{a}$ | 2.7±0.1$^{b}$ | 3.25±0.07$^{a}$ | 2.7±0.1$^{b}$ | 3.33±0.1$^{a}$ |
| Creatinine (mg $dL^{-1}$) | 0.02±0.0$^{c}$ | 0.03±0.04$^{c}$ | 0.05±0.04$^{c}$ | 0.12±0.0$^{b}$ | 0.14±0.06$^{b}$ | 0.22±0.04$^{a}$ | 0.23±0.0$^{a}$ |
| Urea (mg $dL^{-1}$) | 4.85±0.4$^{b}$ | 5.1±0.2$^{b}$ | 3.3±0.4$^{c}$ | 6.7±0.1$^{a}$ | 4.34±0.2$^{b}$ | 7.1±0.3$^{a}$ | 7.8±0.3$^{a}$ |
| Triglycerides (mg $dL^{-1}$) | 274.5±13.6$^{b}$ | 287.1±23.1$^{b}$ | 220.7±14.6$^{c}$ | 458.1±21.4$^{a}$ | 192.7±14.2$^{c}$ | 487.7±17.2$^{a}$ | 197.7±10.6$^{c}$ |
| Total cholesterol (mg $dL^{-1}$) | 184.3±1.8$^{c}$ | 225.7±10.4$^{b}$ | 184.4±13.1$^{c}$ | 221.4±16.5$^{b}$ | 232.7±6.9$^{b}$ | 254.7±15.4$^{a}$ | 246.1±9.8$^{a}$ |
| **Enzymes (IU $L^{-1}$)** | | | | | | | |
| ALT | 29.4±3.42$^{b}$ | 26.5±3.3$^{b}$ | 34.8±4.2$^{b}$ | 21.6±3.7$^{b}$ | 33.6.1±2.4$^{b}$ | 54.7±3.0$^{a}$ | 62.3±1.8$^{a}$ |
| AST | 670±11.5$^{b}$ | 648.6±36.5$^{b}$ | 637.1±7.6$^{b}$ | 598.6±21.2$^{b}$ | 682.1±21.6$^{b}$ | 857±3.5$^{a}$ | 805.1±23.2$^{a}$ |
| LDH | 1099.7±4.2$^{c}$ | 1281.7±78.5$^{b}$ | 1563.1±8.2$^{a}$ | 1270.2±60.4$^{b}$ | 1792.2 ±8.6$^{a}$ | 1681.7±78.5$^{a}$ | 1650.4±13.7$^{a}$ |
| ALP | 49.85±5.3$^{b}$ | 53.3±4.2$^{b}$ | 73.1±3.1$^{a}$ | 75.5±3.8$^{a}$ | 82.3±3.7$^{a}$ | 24.2±4.1$^{c}$ | 21.1±2.5$^{c}$ |
| **Electrolites (mmol $L^{-1}$)** | | | | | | | |
| Cl | 127.45±2.1 | 123.571.9 | 125.3±2.1 | 122.6±2.2 | 124.1±3.6 | 119.571.5 | 129.6±2.3 |
| Na | 152.2±2.4 | 148.671.7 | 152.6±4.1 | 147.6±1.3 | 153.1±3.7 | 146.5±1.2 | 156.5±2.8 |
| K | 2.71±0.6$^{a}$ | 2.2±0.5$^{b}$ | 2.82±0.6$^{a}$ | 1.2±0.3$^{c}$ | 2.81±0.3$^{a}$ | 1.9±0.3$^{b}$ | 2.92±0.4$^{a}$ |

All data points are the average of n =7 with ± SD. $^{a,b,c}$ Statistically significant ($p$<0.05).

**Table 3: Changes on the hematological parameters in rainbow trout blood treated to 10, 20 and 30 ppm propolis and pollen concentrations.**

| Groups / Parameters | Control | Propolis 10 ppm | Pollen 10 ppm | Propolis 20 ppm | Pollen 20 ppm | Propolis 30 ppm | Pollen 30 ppm |
|---|---|---|---|---|---|---|---|
| Total Leucocyte Count ($10^3/mm^3$) | 7.6±0.1$^{c}$ | 7.2±0.2$^{c}$ | 7.4±0.24$^{c}$ | 7.7±0.1$^{c}$ | 13.6±0.24$^{a}$ | 14.5±0.7$^{a}$ | 16.79±0.17$^{a}$ |
| Erytrocyte Count ($10^6/mm^3$) | 0.67±0.1$^{b}$ | 0.70±0.1$^{b}$ | 0.67±0.08$^{b}$ | 0.91±0.03$^{b}$ | 1.2±0.1$^{a}$ | 1.4±0.1$^{a}$ | 1.8±0.02$^{a}$ |
| Hemoglobin (g/dL) | 8.5±0.2$^{b}$ | 8.3±0.1$^{b}$ | 10.0±0.11$^{a}$ | 8.1±0.2$^{b}$ | 11.1±0.42$^{a}$ | 6.9±0.2$^{c}$ | 6.6±0.53$^{c}$ |
| Hematocrit (%) | 26.1±0.6$^{a}$ | 23.9±1.2$^{a}$ | 26.2±0.91$^{a}$ | 27.6±0.4$^{a}$ | 12.9±2.12$^{b}$ | 15.6±1.5$^{b}$ | 11.8±2.25$^{b}$ |
| **Erytrocyte Indexes** | | | | | | | |
| MCV ($\mu^3$) | 238.9±2.3 | 235.4±2.1 | 231.5±2.10 | 240.0±1.9 | 246.5±1.80 | 253.0±2.7 | 244.0±1.60 |
| MCH (μg) | 57.0±1.4$^{c}$ | 57.6±1.7$^{c}$ | 72.9±1.90$^{b}$ | 61.7±1.8$^{c}$ | 99.3±1.60$^{a}$ | 78.4±2.4$^{b}$ | 101.5±1.60$^{a}$ |
| MCHC (%) | 23.8±0.7 | 24.4±0.8 | 27.1±0.70 | 25.7±0.6 | 23.4±0.82 | 26.4±0.7 | 27.1±0.80 |

All data points are the average of n =7 with ± SD. $^{a,b,c}$ Statistically significant ($p$<0.05).



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Most recent studies have shown that natural preventive compounds have gained popularity day by day as some of the widely used synthetic pharmaceuticals and therapeutics might have some undesirable effects (Talas and Gulhan, 2009; Talas *et al*., 2012; Talas and Gulhan, 2013). One can think that certain natural food ingredients would be better and safer than synthetic ones. Many of these compounds, such as plant phenolics, often exhibit antioxidant activities; therefore the addition of these compounds into food products may be helpful to health the of consumers and also to the stabilization of food products (Talas and Gulhan, 2009; Talas et al., 2012). Due to the presence of some of these effective compounds such as flavonoids (flavones and flavanones), phenolic acids and their esters in propolis and pollen extracts, if the positive physiological properties and the non-toxicity of the propolis and pollen samples are proven they could be used as a mild antioxidant and preservative (Talas and Gulhan, 2009; Talas *et al*., 2012). Due to antioxidant and preservative properties of propolis and pollen, both of them may not only prolong the physiological functions of some aquatic living organisms, but also contribute to the health benefit of consumers who consume aquatic animals (Talas and Gulhan, 2009; Talas *et al*., 2012).

The data showed that propolis and pollen have important positive effects on biochemical and hematological parameters at 10 ppm levels, whereas other concentrations appear to be unfavorable for blood of rainbow trouts. Due to antioxidant and preservative properties of propolis and pollen, they may not only prolong the physiological functions of some aquatic living organisms, but also contribute to the health benefit of consumers who consume aquatic animals.

Generally, 10 ppm pollen and propolis extractive concentrations demonsrated the best effect on biochemical and hematological parameters in fish blood. Especially, propolis had been more effective on the hematological parameters in fish according to pollen. Also, propolis extract again had been more effective on most of the biochemical parameters according to pollen extract.

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