Role of propolis on biochemical and hematological parameters of *Oncorhynchus mykiss* exposed to cypermethrin

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Abstract

The protective effects of propolis on biochemical and hematological parameters were appraised in the blood of *Oncorhynchus mykiss* exposed to cypermethrin (CYP). The fish were subjected to three sublethal concentrations of CYP (0.0041, 0.0082 and 0.0123 ppm) and propolis (10 ppm) for 96 h. The samples were analysed to changes in the biochemical parameters, such as the metabolits: glucose, total protein, creatinine, urea, triglycerides, total cholesterol levels and the enzymes: Alanine Aminotransferase (ALT), Alanine Aminotransferase (AST), Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH), amilase, Gamma Glutamyltransferase (GGT) and the electrolites: chloride, sodium, potassium, calcium, phosphate and ferric. Also, hematological parameters including total leucocyte count, granulocytes, agranulocytes and the erytrocyte count: Hemoglobin, hematocrit and the erytrocyte indexes: Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were tested in fish blood. The data of this investigation showed that CYP had a negative effect on the biochemical and hematological variables of the fish; these toxic effects were neutralised by the application of propolis. The results suggest that propolis can be effective in the prevention of CYP-induced toxicity in rainbow trout, especially hematopoiesis.

Keywords: Cypermethrin, Propolis, Biochemical parameter, Hematological parameters, Rainbow trout

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Introduction

Pesticides are extensively used for agriculture but residues can reach aquatic ecosystems, frequently. They are transferred from pytoplankton to fish and human, ultimately (Begum, 2005). In general, pyrethroid insecticides are utilized in place of organochlorine, organophosphorus and carbamates to control different forms of pests and rise yield. These chemicals are probably more toxic for fish and other aquatic organisms and mammals, too (Atamanalp et al., 2002). Cypermethrin, being considerably used pesticide based on pyrethroids, is the most influential pesticide among the pyrethroid types (Velisek et al., 2006).

Cypermethrin ([R,S]α-cyano-3-phenoxybenzyl-2,2-dimethyl(1R,1S)-cis,trans-3-(2,2-dichlorovinyl)cyclopropanecarboxylate) is an insecticide, having quite effective and vast spectrum, generally used to control cotton boll worm (David et al. 2004), and also toxic for many aquatic organisms and fish (Adhikari et al., 2004). The estimation of the ecotoxicological risks in non-target organisms of the ecosystem is based on data of the toxicity and effects of pesticide preparations. Fish are among of non-target aquatic organisms (Velisek et al., 2006). The LC_{50} (96 h) value of this pesticide in the blood of rainbow trout is 0.0082 mg/L. The metabolism and evacuation of cypermethrin in fish is slower than in mammals and birds (Ansari et al., 2011).

To minimize damage and toxic effects caused by pesticides, cells should have improved immune systems consist of antioxidant molecules. When toxic agents are overrun normal level, external antioxidant and protective compounds should be taken. Consequently, the investigation of new antioxidants as promising therapeutic agents is an active field of biochemistry. Various organic types of antioxidants have been worked as possible natural therapeutic and preservative agents. Especially, researchers interested in bee products, which takes a huge part of these natural agents (Kanbur et al., 2009). Propolis is also a natural honey bee product, with various biological activities, including antimicrobial, antiseptic, immunomodulatory, antiinflammatuar, antimutagenic and antioxidant properties (Chu, 2006; Girgin et al., 2006). The compounds of propolis being in the natural environment and structure of plants can go to aquatic areas from agricultural fields via rain water and nutrition. Physiological and biochemical reactions may appear in population or ecosystem. Propolis is a resinous material, which is a product of honeybees (Gunduz et al., 2005). Bees use propolis as a sealer for their hives and more significant to prohibit the decomposition of creatures which have been killed by them after an invasion of the hive. Characteristically, it is a lipophilic material, hard and brittle. Propolis contains 50–70% resins and 10% essential oils, coming from the trees, mixed with 30–50% wax for proper consistency and 5–10% pollen, acquired from being transported in the bees' pollen baskets (Chen et al., 2007). Bioflavonoids, vitamins B1, B2, B6, C, E, and mineral elements manganese, iron, calcium, aluminium and vanadium have been identified in propolis samples Its chemical constituents

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and biological features show variation in various geographical and botanical origin (Sforcin, 2007). Biochemical and hematological parameters of fish are determined as an index of their health status as well. Particularly, fish are mostly used to prediction the influence of environmental constituents due to the sensitivity of their biochemical and hematological parameters under some stress cases (Gulhan et al., 2012). As a sign of stress, the use of hematological and biochemical methods obtain worthy information about physiological reactions occurring against changing environmental states, especially understanding the physiological, biochemical and hematological changes occurring at different concentrations of contituents, to foresee the possible level of threat to life.

The objective of this study was to investigate the protective and therapeutic effects of propolis on the toxicity of cypermethrin in sublethal concentrations on the certain blood parameters of a freshwater fish, *Oncorhynchus mykiss*.

Materials and methods

The rainbow trouts (*Oncorhynchus mykiss*) were buy from Camardı, Ecemis fish farm (Nigde, Turkey). Fish were fed for 15 days in a 8 x 5 x 1.5 m stock pond to be acclimatized. After adaptation period, fish were transferred to 200 L tank filled with water. After acclimatization, fish were randomly assigned to one of 8 experimental treatment groups, each consisting of 64 animals (4 replicate tanks per treatment, each tank containing 8 fish). Airflow was continuous and artificial dry food was provided once daily. Fish used in this study had an average weight of 245.51±5.22 g and length of 29.75±3.81 cm. Physical and chemical properties of water are depicted in table 1. The experiments were applied in accordance with the guidelines for approved by the Committee of Animal Experiments at Cumhuriyet University, Sivas, Turkey.

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

Parameters (ppm)	Before treatment	After treatment
Dissolved oxygen	7.8 ± 0.2	7.6 ± 0.1
Chemical oxygen demand	15.1 ± 0.1	16.2 ± 0.2
Suspended solids	36.8 ± 1.2	40.1 ± 1.7
Calcium	126.0 ± 1.5	114.1 ± 1.1
Sodium	22.4 ± 0.8	19.7 ± 0.7
Chloride	16.0±1.5	18.0±1.4
Total nitrogen	5.8 ± 0.2	6.8 ± 0.3
Hardness ($CaCO_3$)	174.3 ± 3.1	168.2 ± 2.8
Temperature (^oC)	11.5 ± 1	12 ± 0.7
pH	7.6 ± 0.8	7.7 ± 0.6

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Propolis extraction methods may influence its activity, since various solvents solubilize and extract the various compounds. The most general extracts used in biological assays are ethanol, methanol and water (Mani et al., 2006). Chemical composition of propolis is very complex and its composition depends on the local flora. In the study, propolis was collected from a farm at Kocaavsar village in Balikesir, Turkey. Propolis was prepared to 30% in ethanol (30 g of propolis, completing the volume to 100 mL with 70% 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°C until use (Mani et al., 2006). Hematological and biochemical parameters of rainbow trout treated to propolis at different concentrations were survey, and the effects of 10 ppm propolis were outlined, evidencing the conservation role of propolis on hematological and biochemical parameters (Talas and Gulhan, 2009).

In the study, fish exposed to 0.0041 ppm cypermethrin in CYP I group, fish exposed to 0.0082 ppm cypermethrin in CYP II group, fish exposed to 0.0123 ppm cypermethrin in CYP III group, fish treated to 10 ppm propolis in propolis group, fish applied to 0.0041 ppm cypermethrin+10 ppm propolis in CYP I+propolis group, fish applied to 0.0082 ppm cypermethrin+10 ppm propolis in CYP II+propolis group, fish applied to 0.0123 ppm cypermethrin+10 ppm propolis in CYP III+propolis group and fish no applied in control group were used. Each of the experimental groups including 8 fish with four replicates was designed. Each fish was weighted just before the beginning of the study. 0.0041 ppm cypermethrin was applied to the animals in CYP I group for 96 h, 0.0082 ppm cypermethrin was applied to the fish in CYP II group for 96 h, 0.0123 ppm cypermethrin was applied to the rainbow trouts in CYP III group for 96 h, 10 ppm propolis was treated to the animals in propolis group for 96 h, 0.0041 ppm cypermethrin+10 ppm propolis was applied to the animals in CYP I+propolis group for 96 h, 0.0082 ppm cypermethrin+propolis was applied to the fish in CYP II+propolis group for 96 h, 0.0123 ppm cypermethrin+10 ppm propolis was applied to the fish in CYP III+propolis group for 96 h and all of the fish not fed for 12 h before. Fish were fed to Excel Pond trade mark pellet feed during experiments. Fish in the last group were used as control and there were no application. After all of application for 96 hours, fish were anaesthetised with clove oil (40 mg/L) (Mylonas et al., 2005). Then, they were sacrificed in accordance with the guidelines for approved by the Committee of Animal Experiments at Cumhuriyet University, Sivas, Turkey.

After application, 2 mL of blood was withdrawn from caudal vein of fish. Blood samples were transferred to tubes, kept into cooled bath and promptly analyzed. The blood was centrifuged at 3000 x g, at 4ºC for 5 minutes. Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Gamma Glutamyltransferase (GGT), Lactate Dehydrogenase (LDH), amilase activities in plasma were assayed. Glucose, total protein, creatinine, triglycerides, total cholesterol,

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chloride, sodium, potassium, calcium, phosphate and ferric levels in plasma were determined. All of analyses were done with an Olympus Optical Corp. (Shizuoka-ken, Japan), using commercially available kits (Roche).

Blood samples were collected from anaesthetised fish and transferred to tubes. Hematometric parameters were immediately determined. Red blood cell counting was done after 1:200 dilution into Hayem solution. Leucocytes counting was done in blood samples after proper dilution into Turck solution (Blaxhall, 1981). Hemoglobin concentration was determined according to the cyano-methemoglobin procedure (Kit 525- A; Sigma Chemical, St. Louis, MO, USA) (Blaxhall and Daisley, 1973). 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 and the amount of hemoglobin was calculated against to a hemoglobin standard (Azizoglu and Cengizler, 1996). Hematocrit was determined as Wilhelm et al. (Wilhelm et al., 1992). Nonclotted blood was transferred to a microhematocrit capillary, centrifuged at 14.000 g for 5 min and read against to a standart cart.

Hematological and biochemical data were analyzed with SPSS 16.0 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

The changes on biochemical parameters in blood of the fish are summarized in table 2. There were statistically important decreases ($p<0.05$) in the levels of triglycerides, ALT, AST, GGT and important increases ($p<0.05$) in the levels of total protein and chloride in propolis group compared to control group (Table 2). However, there were no significant changes ($p>0.05$) in the levels of glucose, creatinine, urea, total cholesterol, LDH, ALP, amilase and electrolites (sodium, potassium, calcium, phosphate, ferric) in propolis group compared to control group (Table2). The levels of potassium, phosphate and ferric in cypermethrin groups (0.0041, 0.0082 and 0.0123 ppm) compared to control group did not changes statistically significantly ($p>0.05$) (Table). There were statistically significant decreases ($p<0.05$) in the levels of glucose, total protein, triglycerides, chloride and significant increases ($p<0.05$) in the levels of creatinine, urea, total cholesterol, ALP, LDH, amilase, sodium, calcium of cypermethrin groups compared to control group (Table 2). ALP, potassium, phosphate and ferric levels of CYPI+propolis group did not change statistically significantly ($p >0.05$) compared to CYP I group (Table2). The levels of creatinine, urea, total cholesterol, ALT, AST, LDH, amilase, GGT, sodium and calcium of CYP I+propolis group significantly decreased compared to CYP I group ($p<0.05$) (Table 2). But, glucose, total protein, triglyceride and chloride levels in blood serum of CYP I+propolis group significantly increased compared to CYP I group ($p<0.05$) (Table 2). The levels of chloride potassium, phosphate and ferric of CYP II+propolis group compared to CYP II group did not change statistically significantly ($p >0.05$) (Table 2). The levels of

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creatinine, urea, total cholesterol, ALT, AST, LDH, ALP, amilase, GGT, sodium and calcium in serum of CYP II+propolis group significantly decreased compared to CYP II group ($p<0.05$) (Table 2). But, glucose, total protein and triglyceride levels in blood serum of CYP II+propolis group significantly increased compared to CYP II group ($p<0.05$) (Table 2). The levels of ALP, amilase, potassium, phosphate and ferric of CYP III+propolis group compared to CYP III group did not change statistically significantly ($p>0.05$) (Table 2). The levels of creatinine, urea, total cholesterol, ALT, AST, LDH, GGT, sodium and calcium in serum of CYP III+propolis group significantly decreased compared to CYP III group ($p<0.05$) (Table 2). But, glucose, total protein, triglycerides and chloride levels in blood serum of CYP III+propolis groups significantly increased compared to CYP III group ($p<0.05$) (Table 2).

The effects of propolis on the hematological parameters of rainbow trout exposed to different concentrations of cypermethrin are showed in table 3. There were the important rise ($p<0.05$) in agranulocyte level, and the significant decreases ($p<0.05$) in total leukocyte, MCV values of propolis group compared with control (Table 3). However, there were no significant changes ($p>0.05$) in the levels of granulocyte, erytrocyte, hemoglobin, hematocrit, MCH and MCHC. There were no statistically significant changes in the MCH and MCHC levels of rainbow trout exposed to three different concentrations of cypermethrin (0.0041, 0.0082 and 0.0123 ppm) compared to control group ($p>0.05$) (Table 3). But, there were statistically significant decreases ($p<0.05$) in the levels of erytrocyte, hemoglobin, hematocrit while significant increases ($p<0.05$) in the levels of granulocyte in cypermethrin groups compared to control group (Table 3). The levels of MCV, MCH and MCHC of CYP I+propolis group compared to CYP I group did not change statistically significantly ($p>0.05$) (Table 3). The levels of total leukocyte and granulocyte of CYP I+propolis group compared with CYP I group significantly decreased ($p<0.05$) (Table 3). But, agranulocyte, erytrocyte, hemoglobin and hematocrit levels in blood of CYP I+propolis group significantly increased compared to CYP I group ($p<0.05$) (Table 3). The levels of MCH, MCHC and hematocrit in CYP II+propolis group compared to CYP II group did not change statistically significantly ($p>0.05$) (Table 3). The levels of total leukocyte, granulocyte and MCV of CYP II+propolis group significantly decreased compared to CYP II group ($p<0.05$) (Table 3). But, agranulocyte, erytrocyte and hemoglobin levels of CYP II+propolis group significantly increased compared to CYP II group ($p<0.05$) (Table 3). There were no statistically significant changes in levels of MCH, MCHC and hemoglobin of CYP III+propolis group compared to CYP III group ($p>0.05$). There were significant increases ($p<0.05$) in levels of agranulocyte, erytrocyte and hematocrit, but decreases in values of total leucocyte, granulocyte and MCV of CYP III+propolis group compared to CYP III group ($p<0.05$) (Table 3).

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Table 2: Changes on the biochemical parameters in blood serum of rainbow trout applied to propolis and various concentrations of cypermethrin

Parameters \ Groups	Control	Propolis	CYPI	CYPII	CYPIII	CYP I +Propolis	CYP II +Propolis	CYP III +Propolis
Metabolits								
Glucose (mg dL^{-1})	68.5±0.15^{b}	66.1±0.06^{b}	59.3±0.04^{c}	57.3±0.15^{c}	52.1±0.08^{c}	70.7±1.70^{b}	78.2±2.10^{a}	83.1±1.80^{a}
Total protein (g dL^{-1})	2.3±0.09^{b}	2.8±0.08^{a}	1.9±0.07^{c}	1.8±0.06^{c}	1.7±0.08^{c}	2.3±0.07^{b}	2.4±0.01^{b}	2.3±0.11^{b}
Creatinine (mg dL^{-1})	0.12±0.04^{b}	0.14±0.03^{b}	0.72±0.2^{a}	0.77±0.3^{a}	0.79±0.4^{a}	0.32±0.06^{b}	0.26±0.04^{b}	0.45±0.01^{b}
Urea (mg dL^{-1})	4.9±0.2^{c}	5.1±0.2^{c}	5.9±0.2^{b}	6.7±0.2^{a}	6.9±0.4^{a}	5.1±0.2^{c}	6.1±0.3^{b}	6.2±0.3^{b}
Triglycerides (mg dL^{-1})	380.2±16.2^{a}	337.1±13.1^{b}	336.7±2.3^{b}	307.2±2.1^{c}	281.7±14.6^{c}	367.7±14.2^{a}	334.7±10.6^{b}	341.1±11.7^{b}
Total cholesterol (mg dL^{-1})	180.3±11.5^{c}	184.4±12.2^{c}	209.1±7.9^{b}	234.1±8.1^{a}	253.7±10.4^{a}	186.7±6.9^{c}	205.1±9.8^{b}	221.7±7.6^{bc}
Enzymes (IU L^{-1})								
ALT	44.3±4.46^{b}	26.5±6.30^{c}	42.7±5.40^{b}	65.1±3.42^{a}	67.7±4.21^{a}	35.1±2.41^{c}	48.7±1.82^{b}	49.3±3.70^{b}
AST	468.3±8.9^{b}	362.1±7.6^{c}	472.3±10.5^{b}	536.5±13.1^{a}	513.6±16.5^{a}	387.1±21.6^{c}	405.1±23.2^{c}	392.5±10.8^{c}
LDH	784.2±4.2^{c}	772.1±9.1^{c}	831.1±2.8^{b}	895.7±7.5^{a}	912.1±8.2^{a}	792.2 ±8.6^{c}	850.4±13.7^{b}	847.8±19.1^{b}
ALP	54.1±5.91^{c}	53.3±4.22^{c}	64.1±2.61^{b}	74.2±2.90^{a}	78.5±3.13^{a}	60.9±3.72^{b}	62.3±2.54^{b}	72.8±3.40^{a}
Amilase	287.5±14.2^{c}	292.8±13.2^{c}	354.7±10.7^{b}	377.8±12.1^{a}	401.7±11.5^{a}	308.6±16.8^{c}	351.7±14.3^{b}	378.9±10.8^{a}
GGT	6.6±0.42^{b}	5.5±0.44^{c}	6.9±0.32^{b}	8.4±0.56^{a}	8.7±0.40^{a}	5.2±0.63^{c}	6.3±0.52^{b}	6.1±0.61^{b}
Electrolites (mmol $L^{-1)}$								
Chloride	97.6±2.61^{b}	121.5±1.91^{a}	95.8±2.12^{b}	93.1±1.92^{b}	81.7±2.10^{c}	114.3±3.63^{a}	99.1±2.43^{b}	105.7±2.31^{a}
Sodium	151.6±2.53^{c}	148.6±1.75^{c}	166.2±3.28^{a}	171.3±3.44^{a}	181.2±4.10^{a}	156.2±3.72^{c}	169.7±2.85^{b}	162.3±3.50^{b}
Potassium	3.8±0.6	3.2±0.5	4.1±0.6	4.7±0.5	5.2±0.6	3.7±0.3	4.6±0.4	4.8±0.5
Calcium	11.8±0.4^{c}	10.9±0.3^{c}	14.1±0.5^{b}	18.6±0.3^{a}	19.7±0.8^{a}	11.3±0.4^{c}	12.2±0.8^{c}	15.7±0.5^{b}

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Continue table2:								
Phosphate	20.3±1.51	19.8±0.61	20.7±0.81	22.1±1.22	22.4±1.30	20.8±0.71	21.7±0.85	22.1±0.17
Ferric	73.3±4.32	74.2±6.13	65.7±3.12	62.1±2.31	59.7±2.41	68.7±2.72	66.1±2.93	60.1±2.14

All data points are the average of n = 8 with ± SD. [a,b,c] Statistically significant (P<0.05).

Table 3: Changes on the hematological parameters in blood of rainbow trout applied to propolis and various concentrations of cypermethrin

Groups / Parameters	Control	Propolis	CYP I	CYP II	CYP III	CYP I+ Propolis	CYP II+ Propolis	CYP III+ Propolis
Total Leucocyte Count ($mm^3/10^3$)	8.26±0.12[b]	7.72±0.15[c]	8.12±0.17[b]	8.77±0.17[a]	9.13±0.24[a]	7.48±0.13[c]	7.79±0.17[c]	8.23±0.21[b]
Granulocytes (%)	75.4±0.42[c]	77.5±0.46[c]	87.2±0.31[b]	84.2±0.52[b]	98.1±0.60[a]	77.1±0.84[c]	79.2±0.62[c]	85.3±0.56[b]
Agranulocytes (%)	20.6±0.33[b]	26.5±0.43[a]	19.8±0.61[b]	15.8±0.72[c]	13.9±0.37[c]	23.9±0.6[a]	20.8±0.52[b]	21.7±0.48[b]
Erytrocyte Count ($mm^3/10^6$)	1.49±0.06[a]	1.44±0.05[a]	1.16±0.05[b]	0.94±0.02[c]	0.85±0.02[c]	1.35±0.03[a]	1.08±0.08[b]	1.32±0.04[a]
Hemoglobin (g/dL)	8.5±0.22[a]	8.3±0.16[a]	7.2±0.13[b]	6.6±0.28[c]	6.1±0.15[c]	8.2±0.40[a]	7.0±0.54[b]	6.5±0.32[c]
Hematocrit (%)	35.6±1.51[a]	33.9±1.23[a]	27.5±2.62[b]	25.1±1.36[b]	18.2±0.92[c]	32.2±2.17[a]	34.5±2.22[b]	25.6±0.83[b]
Erytrocyte Indexes								
MCV (μ^3)	275.9±2.30[b]	235.4±2.10[c]	237.06±0.86[c]	277.65±3.42[b]	289.7±2.10[a]	237.03±1.80[c]	238.28±1.60[c]	245.09±1.70[c]
MCH (µg)	57.04±1.40	57.6±1.70	62.06±1.90	72.34±2.40	73.8±1.90	58.51±1.60	59.37±1.60	64.7±1.10
MCHC (%)	23.8±0.70	24.4±0.80	26.18±0.80	26.05±0.40	25.61±0.70	24.53±0.82	24.91±0.80	26.29±0.70

All data points are the average of n = 8 with ± SD. [a,b,c] Statistically significant (p<0.05).

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Discussion

The pesticides that are easily transported by the rain from farming fields into lagoons, ponds and rivers, are a source of high toxicity for fish aquaculture (Das and Mukherjee, 2003). In this manner, the non-target aquatic organisms are at high risk of health. Fish blood is being researched progressively in toxicological investigate and environmental monitoring as possible signs of physiological and pathological alters in fishery management and diseases surveys on the blood in unfavorable changes in the water could be reflected in the circulatory system. These studies could be used to indicate the health status of fish as well as water quality (Adhikari et al., 2004).

There have been many studies on reporting the changes in blood parameters rely on various stress states. The pyrethroid insecticide, cypermethrin has been found to cause important morphological and behavioral, biochemical and neurotoxic stress in freshwater fishes by Kumar et al. the earlier reports have shown the increased requirement of energy by freshwater fish under cypermethrin induced toxic stress (Kumar et al., 2009a; Kumar et al., 2009b). Das and Mukherjee showed an increase in the level of blood glucose of fish, *Labeo rohita* exposed to cypermethrin (Das and Mukherjee, 2003). The increased levels of glucose along with simultaneous decline in the level of glycogen in *Heteropneustes fossilis* have been reported under cypermethrin induced stress (Saha and Kaviraj, 2009). Ansari et al. reported that fish exposed to cypermethrin (0.4, 0.8 and 1.2 mg/L for 48 and 72h) showed increasing of frequencies of chromosomal aberration and micronucleus in a concentration-dependent manner (Ansari et al., 2011). Sublethal effects of cypermethrin and carbofuran on hematological parameters and their complete healing were studied in *Labeo rohita* as a role of exposuring time. Erythrocyte count, hemoglobin content and hematocrit values of *Labeo rohita* exposured to sublethal levels of cypermethrin and carbofuran were significantly lower compared with control group (Adhikari et al., 2004). *Rhamdia quelen* was exposed to sublethal concentration of cypermethrin (30% and 45% of the 48-h LC_{50} value of 0.265 ppm) for 2, 4 or 8 days. Biochemical and hematological parameters were analysed in blood. At this concentration, the fish showed behaviorial changes such as hyperexcitability, asphyxia, and widening of mouth and operculum. Results of their work showed that biochemical analyses of serum can be useful to detect incipient cypermethrin intoxication of the shoal (Borges et al., 2007). Recent *in vitro* and *in vivo* studies have shown that cypermethrin produces reactive oxygen species and thus induces oxidative stress. Induction of oxidative stress during its metabolism is also based on the evidence that excitatory events may stimulate ROS production during the cleavage of cypermethrin (Shashikumar and Rajini, 2010).

Survey on the neutralization of pesticides from fish are most significant for the point of view of human health, but there is a shortcoming of knowledge on the recovery of blood cells after exposured to pesticides (Singh and Srivastava, 2010). To eleminate and

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prevent the damages by pesticides, some antioxidant agents can be used. Antioxidant therapy is one of the most important and safe ways to prevent oxidative damage. Synthetic antioxidants are very effective but numerous side effects have been reported from them (Nabavi et al., 2012; Nabavi et al., 2013). Particularly, researchers have been interested in propolis, which plays an important role among these natural agents (Kanbur et al., 2009).

Biochemical and hematological parameters in blood of rainbow trout treated to various concentrations of propolis for 96 h were determined by Talas and Gulhan. Three concentrations (0.01, 0.02 and 0.03 g/L) of propolis extract were used in order to determine the effects of various concentrations of propolis extract by them. Hematological and biochemical parameters of fish treated to different concentraions of propolis were researched, and outlined with the protective influences of 0.01 g/L propolis (Talas and Gulhan, 2009). Long-term effects of propolis application on serum biochemical parameters of rainbow trout (*Oncorhynchus mykiss*) were examinated by Kashkooli et al. Fish were fed on diets including 0, 0.5, 1.5, 4.5 and 9 g propolis/kg diet for 8 weeks to determine the possible toxicity and negative impacts of propolis. At the end of the work, different seric biochemical parameters were tested. On the basis of their data, propolis is a non-toxic material for rainbow trout and its long-term application might not have any side effects (Kashkooli et al., 2011). *In vivo* study has been conducted on healthy women and men in order to research whether daily intake of powdered propolis extract during 30 days has any effects on the following blood parameters and together with routine red blood cell parameters. After 30 days of cure, statistically significant increment in superoxide dismutase activity and alters in some of the red blood cell parameters were defined (Jasprica et al., 2007).

The results of our work are supported by the studies used of propolis administered different concentrations by some researchers (Jasprica et al., 2007; Talas and Gulhan, 2009; Kashkooli et al., 2011). Das and Mukherjee reported that the sublethal exposure works were done for up to 45 days at 1/10 and 1/50 of 96 h LC_{50} of cypermethrin. The 96 h LC_{50} was found to be 0.139 ppm. There were changes in the values of some biochemical and hematological parameters of blood. Extracts of the herb *Datura stramonium* were efficient in countering the toxicity of this pesticide. Their data recommend suggest that sublethal exposuring of cypermethrin alters the biochemical and hematological parameters and exerts stress on the fish. Plant extracts may be utility in counteracting some of these influences (Das and Mukherjee, 2003).

In another study was investigated the effects of propolis on oxytetracycline (OTC)-induced oxidative stress and immunosuppression in fish. OTC had a suppressive effect on specific and nonspecific immune system parameters, such as leukocyte counts, oxidative radical production, total plasma protein and immunoglobulin levels, and phagocytic activity. Treatment with propolis (50 mg per kg^{-1} body weight, orally) reduced the OTC-induced oxidative stress by importantly changing the levels of biochemical

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parameters in tissues. Upon the implementation of propolis, the compressed immune system parameters were significantly increased in fish exposed with OTC. Their present results offer that administration of propolis might reduce OTC-induced oxidative stress and immunosuppression (Yonar et al., 2011).

Yonar et al. (2012) showed preservative effect of propolis on hematological parameters and antioxidant status valuated in the blood and various tissues of carp exposed to sublethal concentrations of chlorpyrifos (0.040 and 0.080 mg/L) for 10 days, and propolis (10 mg per kg of fish weight) was concurrently performed. Their findings demonstrated that chlorpyrifos had a negative effect on the hematological parameters and the antioxidant enzyme systems of the fish; this toxic effect was neutralised by the administration of propolis (Yonar et al., 2012). Talas et al. (2012) determined therapeutic effects of propolis (10 mg/L) on biochemical and hematologic parameters of carp (*Cyprinus carpio*) exposed to arsenic (0.01 mg/L). They emphased that propolis can develop metabolic and physiologic roles of carp blood, after being exposed to arsenic (Talas et al., 2012). The results of our work are in accordance with these previously reported data (Yonar et al., 2011; Gulhan et al., 2012; Yonar et al., 2012; Selamoglu Talas et al., 2012; Kandiel et al., 2013). In this study, the levels of total leucocyte, granulocytes, MCV, MCH and MCHC in cypermethrin groups by increasing of cypermethrin concentration increased and, the levels of erytrocyte, agranulocytes, hemoglobin, hematocrit decreased. Erythrocyte, hemoglobin and hematocrit values in groups of all concentrations of cypermethrin decreased. These cases can be an signs of anemia with subsequent result of inhibition of erythropoiesis in the hematopoietic organs (Johansson-Sjobeck and Larsson, 1978). Furthermore, increases in MCV, MCH and MCHC values showed that anemia was a macrocytic type (Das and Mukharjee, 2003; Dobsikova et al., 2006).

In conclusion; the present study proved that propolis could protect the fish against to harmful effects induced by pesticides, probably due to their free radical scavening availability (Strmack and Braunbeck, 2000). Cypermethrin exposuring caused decreases in the levels of total protein, glucose and triglyceride. The decreases in levels of these parameters in plasma were reported in nephridic syndrome, inflammation and chronic diseases etc. and ascribed to changes in protein synthesis and lipid metabolism (Das and Mukharjee, 2003; Dobsikova et al., 2006). Thus, pesticides might have adversely affected the protein synthesis, lipid and their metabolism in blood of fish used in this study.

Propolis and its compounds are existent in the natural environment and structure of plants. Cypermethrin can go in to aquatic areas from rural and agricultural areas by rain water and nutrients, and has long-term effects in fish and other aquatic organisms. These effects can occur in population or ecosystems with physiological and biochemical reactions. To understand the effects of propolis on the living organisms exposed to cypermethrin should be researched on metric, physiological, hematological and biochemical parameters

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(Banskota et al., 2001). The toxic impacts of cypermethrin on the inhibition of erythropoiesis in the hematopoietic organs and the changes of biochemical parameters have been prohibited by propolis.

Propolis-containing crops have been marketed by the health food stores and pharmaceutical industry (Banskota et al., 2001). Use of propolis go on today in remedies and personal products. It can be used as a mild antioxidant and protective agent because of the presence of efficient constituents such as flavones and phenolic acids in propolis with its positive physiological effects and nontoxic properties. These positive effects of propolis not only may prolong the physiological roles of some aquatic living organisms againts to toxic effects of pesticides, but also may useful to contribute health of human consuming aquatic animals. In the highlight of this study, the importance of propolis for the fish exposed to cypermethrin may significantly effect certain biochemical and physiological roles, notably hematopoiesis can be emphasized. It is known that oxidative stress can be changed hematological parameters such as erythrocyte number, hemoglobin amount, hematocrit value, total leukocytes and biochemical parameters such as glucose, total protein, creatinine, total cholesterol, ALT, AST, ALP.

In this work, we emphasized the impacts of propolis on inhibiting of oxidative stress induced by cypermethrin. In conclusion, these data are directly the evidence of the protection role of propolis on hematological and biochemical parameters against to cypermethrin toxicity in blood of rainbow trout.

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