The determination of different effective concentration of ethanolic extract of bee pollen on biochemical analysis in liver, spleen and heart tissues of rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792)

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Abstract

The aim of this study was to investigate the effective concentration of ethanolic extract of bee pollen on liver, spleen and heart tissues of fish. Bee pollen extract in various concentrations (0.5, 2.5, 5, 10, 20 and 30 ppm) was administered to fish for 96 h under aquarium conditions. The malondialdehyde (MDA) levels, total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI) and levels of total free sulfhydryl groups were investigated in liver, spleen and heart in fish samples. MDA levels in liver, spleen and heart tissues of various concentration groups (2.5, 5, 10, 20 and 30 ppm) decreased (p<0.05) compared to the control group. The highest value of TAS (P<0.05) and the lowest value of TOS (p<0.05) occured in liver and heart tissues of 10 and 20 ppm concentration groups. The lowest levels of OSI were recorded in liver and heart tissues of 10, 20 and 30 ppm concentration groups compared to control group (p<0.05). The highest values of total sulfhydryl groups were recorded (p<0.05) in all tissues of 10 and 20 ppm groups compared with that in the control group. Finally it was observed that in liver, spleen and heart tissues of fish, the antioxidant effects of ethanolic extract of bee pollen depended on concentrations.

Keywords: Pollen extract, Malondialdehyde, Antioxidant status, Oxidant status, Free sulfhydryl, *Oncorhynchus mykiss*

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Introduction

Fish are used as experimental models for analysing pollution in aquatic ecosystems (Kandiel *et al*., 2013). Oxidative stress is the result of imbalance between reactive oxygen species (ROS) and antioxidants in organisms (Talas and Gulhan, 2013). Also, toxic biochemical events may cause various cellular injuries and diseases (Marghitas *et al*., 2009). Most of investigations have recently emphasized the antioxidant effects of various nutritional products. Furthermore, the antioxidative capacities of phenolic compounds of natural products have been tested. The high capacities of phenolic substances to neutralize the ROS are strongly releated to their structural composition, such as the combined double bonds and the number of hydroxyl groups in the aromatic ring, mostly attributed to flavonoids and cinnamic acid derivatives (Silva *et al*., 2000; Nijveldt *et al*., 2001). Honey bee products, especially rich in flavonoids, have been the focus of investigations (Saric *et al*., 2009; Marghitas *et al*., 2009). Among them, special attention should be paid to the floral pollen used for many years as a beneficial dietary supplement (Leja *et al*., 2007). Bee-collected pollen, which is generally gathered from pollen sample from various plants, can be considered as a potential source of energy and nutrients for human (Kroyer and Hegedus, 2001). Fish are very sensitive against changes in their environment. As a result of these changes may affect the certain tissues and organs of fish, including liver, spleen and heart (Dastan *et al*., 2014; Aldemir *et al*., 2014) Fish liver is the primary organ associated with the biotransformation of organic contaminants and metals. This organ is very sensitive against organic and inorganic agents which is releated to the environment itself and other organs (Stori *et al*., 2014). Spleen is an erythropoietic tissue involved in the synthesis of new erythrocytes and is a reservoir of primary hemopoietic organs. Spleen is the unique organ in fish to trap antigens (Balamurugan *et al*., 2012). Contraction and relaxation in the working of fish heart are a result of the complex interaction of many individual cells connected together by specialized adhesion structures. The heart allows generating pressure to pump blood around the body. This organ is important for adaptation to environmental conditions such as fluctuating, temperatures, oxygen and pH (Galli and Richards, 2012). The most widely used assay for lipid peroxidation is the malondialdehyde (MDA) analyses (Gulhan *et al*., 2012). Phenolic compounds such as phenolic acid, flavonoids and tannins are thought to be important subscribers of the antioxidant capacities of foods (Marghitas *et al*., 2009; Segvic-Bubic *et al*., 2013). After treatments with some antioxidants, the assays of total antioxidant status (TAS) (Erel, 2004) and total oxidant status (TOS) can reflect the biochemical changes in

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tissues (Erel, 2005). The changing of protein conformation correlates with exposure to environmental stress of functional groups such as sulfhydryl groups and hydrophobic groups (Dastan *et al*., 2014). However, according to the changes of total sulfhydryl groups (T-SH) in contents of proteins, we can understand more about the changing of conformation and formation of disulfide bonds (Ko *et al*., 2007).

The aim of the present work is to examine the effects of various concentrations of bee pollen extract (0.5, 2.5, 5, 10, 20, and 30 ppm) on biochemical parameters (MDA, TAS, TOS, and total free sulfhydryl groups) in liver, spleen and heart tissues of rainbow trout, *O. mykiss*.

Materials and methods

Animals and experimental design

Rainbow trout (*O. mykiss*) with an average weight of 248.54±5.12 g were obtained from Camardi, Ecemis fish farm in Nigde, Turkey just before the beginning of the study. They were then transferred to a research station in Nigde University under optimum conditions in containers (dimension of 8x5x1.5 m) and acclimated for 15 days. During the experiment, fish were nourished with Pinar pellet commercial food (45% protein, 19% fat, 3% crude fiber) once a day. Physical and chemical parameters of water were analysed for study groups (Table 1). Seven experimental groups, each consisting eight animals, were used in the present study with four replicates. At the end of the treatment period, we randomly sampled 2 fish from each tank for assessment of biochemical parameters (4 replicate tanks per treatment, each tank containing 7 fish); thus 8 fish (4 replicates) were sampled per treatment. As a result, each experimental group included totally 8 animals with four replicates. The fish were administered 0.5 ppm bee pollen ethanol extract in 6 groups: group I, 2.5 ppm;, group II, 5 ppm; group III, 10 ppm; group IV, 20 ppm; group V 30 ppm; and group VI untreated fish as control group were used for 96 h (Talas and Gulhan, 2009). Then, they were sacrificed in accordance with the guidelines approved by the Committee of Animal Experiments at Cumhuriyet University, Sivas, Turkey.

Preparation of bee pollen ethanol extractive solution

Bee pollen was obtained from a farm at village Kocaavsar in Balikesir, Turkey and diluted to 30% in ethanol. It was kept in dark at room temperature and moderately shaken for one day. Afterward, the ethanolic extracts were filtered twice, dried and stored in sealed bottles at 4°C until used (Marghitas *et al*., 2009).

Preparation of tissues for biochemical analyses

After application for 96 hours, fish were anaesthetised with 40 mg/L of clove oil (Mylonas *et al*., 2005). Afterwards; liver, spleen and heart tissues of fish were removed and stored at -80 °C until

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analysed. The tissues were separated into two parts for determination of MDA levels and the other biochemical parameters. Tissues were weighed and then homogenized in 100 mL of 2 mM phosphate buffer, pH 7.4. Samples were centrifuged at 12,000 g for 10 min at 4°C and then supernatants were kept in the deep freeze at -80°C until analysed (Selamoglu Talas *et al*., 2014). Supernatants were used for determination of TAS, TOS, OSI and total sulfhydryl groups. The second parts of tissue homogenates were used for MDA analyses (Selamoglu Talas *et al*., 2014). Tissues were washed three times with ice-cold 0.9% NaCl solution and homogenized in 1.15% KCl. The homogenates were assayed for MDA, the last product of lipid peroxidation (Selamoglu Talas *et al*., 2014).

Malondyaldehyde (MDA) levels

The malondialdehyde (MDA) amount indicated that data were expressed as nmol/g wet tissue. A marker of lipid peroxidation was assayed in the form of thiobarbituric acid reacting substances (TBARS) by using Esterbauer and Cheesman's method (Esterbauer and Cheeseman, 1990).

Total antioxidant status (TAS)

Total antioxidant status in serum of tissues were tested by using the Erel method (Relassay, Turkey). The hydroxyl radical was used in this method. It is the most potent radical for biological molecules. The test has very sensitive values of <3%. As a result , data were expressed as millimoles of Trolox equivalent per liter (mmol Trolox equiv./L) (Erel, 2004).

Total oxidant status (TOS)

TOS in serum of tissues was analysed by using automatic measurement method developed by Erel (Relassay, Turkey). Oxidants in the tissue samples oxidize the ferrous ion–o-dianisidine complex to ferric ion. The test was calibrated with hydrogen peroxide and the obtained results were meaned in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Equiv./L) (Erel, 2005).

Oxidative stress index (OSI)

The ratio of TOS to TAS was measured to be the OSI. The OSI level was calculated according to the following formula: OSI (arbitrary unit)=[TOS (μmol H_2O_2 Equiv./L)/TAS (mmol Trolox Equiv./L)]x100 (Erel, 2004; Erel, 2005).

Total free sulfhydryl groups

Free sulfhydryl groups of tissue patterns were determined according to the method of Ellman (Ellman, 1959) followed by Hu *et al*. (1993). Following incubation for 15 min at room temperature, absorbance was tested at 412 nm by spectrophotometer (Perkin Elmer, Lambda 25). Samples and reagent blanks were subtracted. The concentration of sulfhydryl groups was calculated by using reduced glutathione as standard of free sulfhydryl groups

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and the results were expressed as mmol/mg protein (Sanz *et al*., 2013).

Statistical analysis

Data of biochemical parameters were tested by SPSS 16.0 for Windows by using nonparametric Kruskal-Wallis test. Differences between ranks were determined using Mann-Whitney test in which the significance level was defined as $p< 0.05$.

Results

The changes in physical and chemical parameters of water during the present study have been shown in Table 1.

The effects of bee pollen extracts in different concentrations on MDA levels, TAS, TOS, OSI and total free sulfhydryl groups in liver, spleen and heart tissues of fish have been shown in Tables 2, 3 and 4. Levels of MDA in tissues of fish administered 0.5 ppm pollen extract did not change ($p>0.05$) compared to that in the control group (Tables 2,3 and 4), but there were statistically significant decreases ($p<0.05$) in MDA levels of liver, spleen and heart tissues of 2.5, 5, 10, 20 and 30 ppm pollen groups.

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

Parameters	Before treatment	After treatment
Dissolved oxygen (ppm)	7.6 ± 0.6	7.4 ± 0.3
Chemical oxygen demand (ppm)	13.1 ± 0.4	15.5 ± 0.8
Suspended solids (ppm)	37.6 ± 1.5	41.1 ± 1.2
Calcium (ppm)	132.0 ± 1.8	109.1 ± 1.5
Sodium (ppm)	24.4 ± 0.4	17.7 ± 0.3
Chloride (ppm)	15.0±1.2	16.0±1.8
Total nitrogen (ppm)	5.3 ± 0.5	6.2 ± 0.7
Hardness ($CaCO_3$) (ppm)	179.3 ± 3.6	163.2 ± 2.3
Temperature (^oC)	12.5 ± 1.6	11 ± 0.3
pH	7.6 ± 0.1	7.6 ± 0.1

Table 2: Changes on the biochemical parameters in liver tissues of fish treated to various concentrations of pollen extracts.

Parameters	Groups and concentrations						
	Control	0.5 ppm (Group I)	2.5 ppm (Group II)	5 ppm (Group III)	10 ppm (Group IV)	20 ppm (Group V)	30 ppm (Group VI)
MDA (nmol/g wet tissue)	29,13±2,56^a	25,57±0,85^a	17,68±1,49^b	9,87±0,46^c	10,86±0,42^c	11,02±0,99^c	10,90±0,73^c
TAS (mmol Trolox equivalent/g P)	1,75±2,20^c	1,97±2,00^c	1,81±2,41^c	2,31±1,14^b	2,92±2,41^a	2,72±1,20^a	2, 54±2,35^b
TOS (µmol H_2O_2 (equivalent/L)	4,90±0,25^a	4,04±0,17^b	3,93±0,35^b	4,10±0,16^b	3,14±0,31^c	3,24±0,10^c	3,11±0,13^c
OSI (arbitrary units)	0,28±0,21^a	0,20±0,14^b	0,21±0,19^b	0,18±0,07^b	0,10±0,05^c	0,13±0,06^c	0,12±0,01^c
Total free sulfhydryl group (mmol/mg P)	2,66±0,24^b	1,91±0,35^c	2,07±0,33^c	2,65±0,21^b	3,94±0,58^a	3,79±0,18^a	2,73±0,15^b

All data show the average of n=8 with ±SD. ^{abc}statistically significant ($p<0.05$).

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TAS levels of all tissues with 0.5 and 2.5 ppm pollen administration were stable ($p>0.05$) compared with control group (Tables 2,3 and 4). But, TAS levels in all of tissues of 5,10, 20 and 30 ppm polen groups significantly increased compared to control group ($p<0.05$) (Tables 2,3 and 4). The levels of TOS in liver, spleen and heart tissues in all of the pollen concentrations significantly decreased compared to that in the control group ($p<0.05$) (Tables 2, 3 and 4). OSI levels in liver and spleen tissues in all concentrations of pollen extracts decreased compared to levels in the control group. But, OSI levels in heart tissues of 0.5 and 2.5 ppm pollen concentrations were stable compared to that in the control group. Levels of the total free sulfhydryl group in liver tissues of 0.5 and 2.5 ppm pollen groups decreased statistically ($p<0.05$) compared to the control group (Table 2). But levels of total free sulfhydryl groups in liver tissues of 5 and 30 ppm pollen groups did not change ($p>0.05$) compared to control group (Table 2). The levels of total free sulfhydryl group in liver tissues significantly increased in 10 and 20 ppm pollen groups compared to that in the control group (Table 2). Level of sulfhydryl group was stable with 0.5 ppm pollen administration ($p>0.05$) but levels of sulfhydryl group in spleen tissues of other concentrations of pollen extracts increased compared to control group ($p<0.05$) (Table 3). Total free sulfhydryl levels in heart tissues of groups treated with 5, 10, 20 and 30 ppm pollen extracts significantly increased compared to control group ($p<0.05$) (Table 4).

Table 3: Changes on the biochemical parameters in spleen tissues of fish treated to various concentrations of pollen extracts.

Parameters	Groups and Concentrations						
	Control	**0.5 ppm (Group I)**	**2.5 ppm (Group II)**	**5 ppm (Group III)**	**10 ppm (Group IV)**	**20 ppm (Group V)**	**30 ppm (Group VI)**
MDA (nmol/g wet tissue)	13,39±0,94^{a}	11,82±0,52^{a}	8,80±0,27^{b}	7,69±0,62^{b}	5,64±0,16^{c}	6,06±0,33^{c}	7,38±0,45^{b}
TAS (mmol Trolox equivalent/g P)	2,01±0,99^{c}	1,98±4,83^{c}	2,04±4,54^{c}	2,39±0,81^{b}	2,71±1,19^{a}	2,47±3,91^{a}	2,59±3,47^{a}
TOS (µmol H_2O_2 (equivalent/L)	6,24±0,70^{a}	5,04±0,20^{b}	4,31±0,81^{b}	4,29±0,16^{b}	2,26±0,70^{c}	3,77±0,30^{c}	4,25±0,12^{b}
OSI (arbitrary units)	0,31±0,21^{a}	0,25±0,14^{b}	0,21±0,13^{b}	0,17±0,07^{b}	0,09±0,05^{c}	0,15±0,06^{b}	0,16±0,01^{b}
Total free sulfhydryl group (mmol/mg P)	1,95±0,24^{c}	1,80±0,90^{c}	2,48±0,33^{b}	2,67±0,50^{b}	3,74±0,27^{b}	3,93±0,36^{a}	2,80±1,14^{b}

All data show the average of n=8 with ±SD. ^{abc}statistically significant ($p<0.05$).

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Table 4: Changes on the biochemical parameters in heart tissues of fish treated to various concentrations of pollen extracts.

Parameters	Groups and Concentrations						
	Control	0.5 ppm (Group I)	2.5 ppm (Group II)	5 ppm (Group III)	10 ppm (Group IV)	20 ppm (Group V)	30 ppm (Group VI)
MDA (nmol/g wet tissue)	13,55±0,54^{a}	12,91±0,48^{a}	9,93±0,40^{b}	8,64±0,28^{b}	5,43±0,60^{c}	6,10±0,64^{c}	8,43±0,37^{b}
TAS (mmol Trolox equivalent/g P)	1,85±1,22^{c}	1,83±0,58^{c}	1,76±0,20^{c}	2,07±0,83^{b}	2,78±0,30^{a}	2,69±0,55^{a}	2,13±1,19^{b}
TOS (μmol H_2O_2 (equivalent/L)	5,44±0,23^{a}	4,86±0,17^{b}	4,17±0,13^{b}	3,75±0,20^{b}	3,80±0,16^{b}	3,89±0,10^{b}	3,12±0,12^{c}
OSI (arbitrary units)	0,27±0,16^{a}	0,26±0,14^{a}	0,23±0,13^{a}	0,18±0,07^{b}	0,13±0,25^{b}	0,14±0,16^{b}	0,14±0,07^{b}
Total free sulfhydryl group (mmol/mg P)	1,14±0,10^{c}	1,23±0,06^{c}	1,20±0,19^{c}	1,61±0,09^{b}	1,85±0,10^{a}	1,97±0,14^{a}	1,92±0,12^{a}

All data show the average of n=8 with ±SD. ^{abc}statistically significant (p<0.05).

Discussion

Aerobic organisms under physiological situations permanently generate ROS as products of oxidative metabolism. Mostly, high rates of ROS damage cells and cause DNA injury, enzyme denaturation and structural changes of proteins as well as peroxidation of polyunsaturated fatty acids (PUFAs) leading to pathologies and alteration of development (Duran and Selamoglu Talas, 2009). Since fish contain high concentrations of n-3 HUFA, they are important to lipid peroxidation and tissue damage resulting from lipid peroxidation (Mercan *et al.*, 2013). Antioxidant therapy is the most important treatment against oxidative damage. Synthetic antioxidants are very effective but numerous side effects have been reported from them (Nabavi *et al*., 2012; Nabavi *et al*., 2013). In the present study, there were statistically significant (p<0.05) decreases in MDA levels of liver, spleen and heart tissues of all experimental groups applied to various concentrations of pollen extract compared to control group (Tables 2, 3 and 4). The antioxidant effect of honeybee-collected pollen has been recognised as free radical scavenger and lipid peroxidation inhibitor as previously reported by Almaraz-Abarca *et al*. (2004). The study of Giannenas *et al*. (2012) showed theness effective of two phytogenic feed additives, rich in carvacrol (12 g/kg) and in thymol (6 g/kg) in decreasing MDA levels of rainbow trout (*Oncorhynchus mykiss*). In another study, the efficacy of green tea (*Camellia sinensis*) was investigated on growth performance, immune and antioxidant systems and cytokine gene expression in tissues of rainbow trout. Green tea was supplemented at 20, 100, and 500 mg kg^{-1} diet and fed to fish (average weight: 23,5 g) for 35 days. Lower production of lipid peroxidation

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and higher activity of superoxide dismutase in fish receiving a medium dose of green tea were determined (Nootash *et al*., 2013). Hamre *et al*. (2010) reported that various natural antioxidants (rosemary extract, crystalline ascorbic, acid tocopherol mix, spermine etc.) showed significant effects in experimental fish feed. The researchers have referred that potent antioxidant properties have also been effective in removing free radicals and show protective effects against lipid peroxidation in cell membranes (Hamre *et al*., 2010). Sahin *et al.* (2014) suggested that supplementation of different doses of lycopene to high density stressed fish causes a dose dependent decreased level of MDA as well as increased activity of antioxidant enzymes (GSH-Px, SOD and CAT) in the liver. Their results indicate the important role of lycopene in the reduction of oxidative stress (Sahin *et al*., 2014). The results of our work are supported by the studies used of various antioxidant molecules by some researchers (Hamre *et al.*, 2010; Giannenas *et al*., 2012; Nootash *et al*., 2013; Sahin *et al*., 2014).

There are many evidences of effectiveness of various natural antioxidants in removing free radicals and their protective effects and antioxidant properties. This study opens a new perspective on the test of pollen biological properties, mainly with respect to biochemical parameters in rainbow trout. Taken together, these data support the hypotheses that pollen may possess antioxidant properties that could influence serum oxidant and antioxidant balance. Because of the number of different antioxidants in plasma, serum or other biological samples it is difficult to measure each antioxidant separately. Some methods have been developed to determine the antioxidative properties of different biological matter (Sekhon-Loodu *et al*., 2013). The measurement of TAS can only reflect the antioxidative status of tissues (Gulhan *et al*., 2014). According to TAS measurements of tissue serum, 10 mg/L treatment group has the highest TAS level among six concentrations (0.5, 2.5, 5, 10, 20 and 30 mg/L) of pollen extract. In 10 and 20 ppm pollen groups, pollen can behave as the best antioxidant for these concentrations. Results of the present study demonstrate similarity with data of previous works (Talas and Gulhan, 2013; Gulhan *et al*., 2014).

The changes on biochemical and hematological parameters in blood of samples treated with 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 extracts were used to determine the effects of various concentrations of propolis extracts in that study. Hematological and biochemical parameters of rainbow trouts treated to various concentraions of propolis were investigated, and outlined with the preventive effects of 0.01 g/L propolis (Talas and Gulhan, 2009). Long-term effects of propolis

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administration on serum biochemical parameters of rainbow trouts (*O. mykiss*) were investigated by Kashkooli *et al*. (2011) . Fish were fed on diets containing 0, 0.5, 1.5, 4.5 and 9 g propolis/kg diet for 8 weeks to determine the possible toxicity and side effects of propolis. At the end of the experiments, various seric biochemical parameters were analysed. On the basis of their findings, propolis is a non-toxic substance for rainbow trouts and its long-term administration might not have any side effects (Kashkooli *et al*., 2011). Pollen contains antioxidant properties that could influence serum oxidant and antioxidant balance. We determined TAS, TOS in fish tissues treated with various concentrations of pollen. To measure separately the each of different antioxidants in plasma, serum or other biological samples is difficult for each antioxidant molecule. Therefore, several methods have been developed to determine the antioxidative capacity of various biological samples (Sanz *et al*., 2013). It has been shown that 10 and 20 ppm pollen extracts are more effective on TAS levels in liver, spleen and heart tissues. Generally, TOS levels in liver, spleen and heart tissues of fish treated to 10 and 20 ppm concentrations of pollen extracts decreased. These results are related to flavonoid compounds of pollen that scavenge free radicals and inhibit oxidative damage in liver, spleen and heart tissues. The data of our study showed similarity with study by Saric et al. (Saric *et al.*, 2009).

Chenga *et al.* (2013) investigated effects of pollen extract on DNA damage caused by oxidative stress. It was determined that pollen extract has radical scavening effect for protecting DNA from damage of free radicals. It is related to fenolic and flavonoid compounds of pollen (Chenga *et al*., 2013). It has been reported that lipoic acid is a potential therapeutic agent due its antioxidant properties. The effects of lipoic acid on different organs (gills, brain, muscle and liver) of *Corydoras paleatus* were considered. Lipoic acid (70 mg/kg of body mass) was added to a commercial fish diet, and increases in TAS levels occured (Monserrat *et al*., 2008). Ural investigated the potential ameliorative effects of lycopene against oxidative stress in liver, kidney and gill tissues of carp. Oxidant-antioxidant status of these tissues were analysed after treatment of lycopene. The findings of that study demonstrated that oxidant effect was neutralised by the administration of lycopene. The results suggest that lycopene (10 mg kg^{-1}) can be effective in the protection against oxidative damage in fish (Ural, 2013). The antioxidant capacity related to the phenolic composition of monospecific honeybee-collected pollen extract of the *Prosopis juliflora* from Durango, Mexico, was evaluated in vitro and in vivo by quantification of thiobarbituric acid reactive substances (TBARS). The results obtained suggest that pollen of *P. juliflora* is an important source of flavonoids, which can be considered as

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natural antioxidants (Almaraz-Abarca *et al*., 2007).

According to data, OSI values of both liver and spleen tissues of fish in all experimental groups decreased (p<0.05) after the application of pollen extract compared with the control group (Tables 2 and 3). Moreover, the strong antioxidant effects of 10, 20 and 30 ppm pollen extracts was carried out by decreasing TOS and OSI values. Various forms of oxidative damage such as thiolation, methylation, and carbonylation can occur in protein molecules. The first two of the mentioned damages are reversible and also, may affect some antioxidant functions. The carbonyl formation in proteins can lead to enzymatic degradation of the proteins. Research indicated that protein carbonylation increased with oxidative stress (Valavanidis *et al*., 2006; Almroth *et al*., 2008). Since the sulfhydryl groups of proteins are exposed, a lot of disulfide bonds are formed due to the increase in interaction of the interior and exterior amino acids (Raikos *et al.*, 2007). Ko et al. investigated changes of sulfhydryl groups and conformation of actomyosin (5 mg/mL) extracted from muscle tissue of tilapia (*Orechromis niloticus*) administered thermal treatments and found an increase in sulfhydryl groups with an increase in therapeutic concentrations (Ko *et al.*, 2007). The antimutagenic and antioxidant activities of captopril, cysteine, and glutathione were evaluated on concentration-dependent inhibitory effects against: the mutagenicity of 2-amino-3-methylimidazo quinoline, an indirect mutagen; and *N*-methyl-*N*′-nitrosoguanidine, a direct mutagen toward *Salmonella typhimurium* TA98 and TA100. These data suggested that the bioactive properties of biological thiols might contribute to their antimutagenic activities as well as regulation on activities of antioxidant enzymes (Duh *et al*., 2009). In our study, total free sulfhydryl levels in liver and heart tissues of fish treated with 0.5 and 2.5 ppm pollen extract significantly decreased. Generally, levels of total free sulfhydryl groups increased with the application of 10 and 20 ppm pollen extracts and therefore, pollen plays a role as antioxidant agent. Our data have shown conformity with that of other researchers (Ko *et al*., 2007; Van Nguyen *et al*., 2011). Pollens of plants are the most important source of proteins and free amino acids for bees and humans (Gulhan *et al*., 2014). Increasing sulfhydryl levels of fish in 10 and 20 ppm pollen groups may be due to the proteins and the free amino acids in pollen.

This work is the first study to determine the effective concentration of pollen on biochemical parameters in tissues of rainbow trout. This study aimed to determine the effective concentrations of pollen extract as an antioxidant agent on biochemical parameters in liver, spleen and heart tissues of rainbow trout. Antioxidant activities of honeybee products are very

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important for biological systems. Due to antioxidant properties of honeybee products, they may prolong the physiological and metabolic functions of some aquatic living organisms. As a result, the present study shows that pollen supports the specific and nonspecific antioxidant systems in rainbow trout. Also, pollen treatment may provide protective effects against the oxidative stress induced by environmental effects. Therefore, pollen administration may activate the cellular antioxidant systems of rainbow trout and pollen may be used as a potent protective agent. However, further investigations are necessary to elucidate the exact mechanism of protective effects and the potential usefulness of this product as an antioxidant. For the first time, the present work investigates the effects and useful concentration of pollen on biochemical parameters in some tissues of fish. Due to antioxidant and preservative properties of pollen, this study suggests that pollen may not only prolong the physiological functions of some aquatic living organisms, but also contribute to the health benefits of consumers who consume fish as a food. We suggest that pollen may be used as a protector of fish tissues. Also, the results of this work will shed light on new research in the future and contribute to scientific literature. The results suggest that pollen may possess antioxidant properties that could influence serum oxidant and antioxidant balance in treated fish.

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