Effects of propolis on some blood parameters and enzymes in carp exposed to arsenic

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Abstract

The purpose of this study was to investigate the therapeutic effects of natural products like propolis on biochemical and hematologic parameters in carp (*Cyprinus carpio*, Linnaeus 1758) exposed to arsenic. In this study fish were exposed to 0.01 mg/L arsenic and 10 mg/L propolis for seven days. Our results indicated that triglyceride, urea, total cholesterol, cobalt, ALT (alanine amino transferase), AST (aspartate amino transferase), LDH (lactate dehydrogenase) values increased when exposed to arsenic (P<0.05) but reduced by combination of arsenic and propolis (P<0.05). Granulocyte, erythrocyte, hemoglobin, hematocrit values were decreased by use of arsenic in comparison to control group. These parameters increased in arsenic+propolis group (P<0.05). In addition levels of leucocyte, agranulocyte, MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin) and MCHC (mean corpuscular hemoglobin concentration) increased in the arsenic group (P<0.05) and decreased in arsenic+propolis group (P<0.05). We can say that propolis can improve biochemical and hematologic functions of common carp blood, after being exposed to arsenic.

Keywords: Antioxidant, Arsenic, Blood parameter, *Cyprinus carpio*, Hematological parameter, Propolis

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Introduction

Arsenic is an element that is present at low concentrations everywhere such as in air, soil and water (Gupta et al., 2005; Rana et al., 2010). Compounds of arsenic, concentrated in the environment, as a result of natural or anthropogenic sources, become a major concern for environmental and occupational health (Gupta et al., 2005; Rana et al., 2010).

Arsenic exists in different chemical forms and oxidation status which influence its bioavailability and toxicity (Ventura-Lima et al., 2009). Inorganic compounds are more toxic than organic forms, despite differences that exist between the effects of arsenite (AsIII) and arsenate (AsV). Arsenite can bind to sulfphydryl (–SH) groups in proteins while arsenate interferes with phosphorylation reactions (Ventura-Lima et al., 2009). In the aquatic environment, arsenic exists either as, AsIII and AsV forms which are inter converted through redox and methylation reactions (Hughes, 2002; Kavitha et al., 2010). These types of arsenic can accumulate in many aquatic organisms which may catalyse the oxidation of arsenite to arsenate and promote the formation of methylarsines through biomethylation reaction (Hughes, 2002; Kavitha et al., 2010). One of the most toxic of these is arsenic trioxide and it is one of the arsenites (inorganic forms of arsenic in the trivalent state [AsIII]) (Gupta et al., 2005; Ventura-Lima et al., 2009; Rana et al., 2010).

Trivalent arsenic toxicity may be through that directly connecting with –SH groups, or indirectly through production of reactive oxygen species (ROS) (Kalia et al., 2007; Banerjee et al., 2009). Eventually, oxidative stress may occur partially with arsenic toxicity (Kalia et al., 2007; Banerjee et al., 2009). Although arsenic is not biomagnified through the food chain, bioconcentration has been observed in various aquatic organisms such as a fish (Schlenk et al., 1997). Freshwater fish uptake arsenic not only through diet by benthic-feeding but also with waterborne across the gill (Pedlar et al., 2002a,b).

Prevention of heavy metal toxicity may be accomplished by either reducing the possibility of metal interacting with critical biomolecules and inducing oxidative damage, or by bolstering the cells antioxidant defenses through endogenous and exogenous supplementation of antioxidant molecules (Kalia et al., 2007). To minimize toxic effects and damages caused by arsenic, cells have improved defense systems which include antioxidant molecules. When toxic agents against the natural protective systems overrun, exogenous antioxidative and protective compounds must be taken (Devillers et al., 2001).

Eventually, to search new antioxidants as potential therapeutic agents is an active field of biochemistry. In recent years, several organic forms of antioxidant molecules have been studied as preventive agents and natural therapeutics. In particular, researchers have been interested in propolis, which plays an important role among these natural agents (Kanbur et al., 2009). Propolis (bee glue) is a natural dark-coloured, resinous sticky substance produced by honey bees by mixing their own waxes with resins collected from plants, and is used as a sealant and sterilant

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in their nests (Sforcin et al., 2000; Moreira et al., 2008). Propolis has been used since ancient times as a medicine owing to those biological properties as an antifungal, antiprotozoal, antimicrobial, and antiviral agent (Sforcin et al., 2000; Moreira et al., 2008).

The fish products have attracted a source of high amounts of significant nutritional components and considerable source of protein in the human diet (Ozogul et al., 2005; Duran and Talas, 2009; Yousefian et al., 2011). The main aim of the present study was investigating the effects of propolis on hematological and blood biochemical in carp (*Cyprinus carpio*, Linnaeus 1758) exposed to arsenic (As_2O_3).

Materials and methods

Criteria of water

The carp were obtained from Azatli Dam Lake (Nigde, Turkey). Fish were fed for 15 days in an 8 x 5 x 1.5 m stock pond to be acclimated. They were transferred to a 200 L tank filled with water. Airflow in the tank was continuously provided and fish were given artificial dry food once daily. Physical and chemical properties of the water are depicted in table 1.

Table 1: Some parameters of the water used in the experiment

Parameters (mg/L)	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)	18.5 ± 1	20 ± 0.5
pH	7.5 ± 0.1	7.6 ± 0.1

Experimental design

In this work, a total of 32 healthy fish were used and thirty-two carp were divided into four groups, each consisting of eight animals. The average mass of fish was determined as 500-600 g. Fish in the first group were used as a control and there was no application. 10 mg/L propolis (Talas and Gulhan, 2009) was treated to the carp

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in the second group for one week and they were not fed for 12 h before; 0.01 mg/L (Schlenk et al., 1997) of arsenic (As_2O_3) (from Aldrich, 98 % purity), was treated to the fish in the third group for one week and they were not fed for 12 h before; both 10 mg/L propolis and 0.01 mg/L arsenic (As_2O_3) were applied to the animals in the fourth group for one week and they were also not fed for 12 h before.

Preparation of propolis extractive solution

In the present study, propolis was collected from a farm at village Kocaavsar 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 one day at room temperature. After that the propolis extract was filtered twice, dried and stored in sealed bottles at 4°C until use (Mani et al., 2006).

Preparation of blood samples for biochemical analyses

Blood draw operation of fish non-applied anaesthetic material was carried out from caudal vena by cutting with a sharp knife 1-2 cm behind the tail fin and taking about 2 mL of blood to glass tubes. For fear of clotting, blood draw operation was carried out in a very short time like 45 sec. Blood samples in the glass tubes were centrifuged at 3500 g for 10 min and serum was transfered into eppendorfs. Afterwards amounts of ALP (alkaline Phosphatase), AST (aspartate aminotransferase), ALT (alanine aminotransferase), LDH (lactate dehydrogenase) and amylase activities were assayed. Also levels of glucose, total protein, creatinine, triglycerides, total cholesterol, urea, albumin, globulin, sodium, potassium, calcium, phosphates and cobalt in serum were determined by autoanalizer. All these analyses were done (Olympus Optical Corp., Shizuoka-ken, Japan) using commercially available kits (Roche).

Hematological analyses

Blood samples were collected prior to anesthetizing fish to prevent hemolysis (McKnight, 1966) and they were transferred into tubes. Hematometric parameters were immediately determined to red blood cell counting after 1:200 dilution into Hayem solution was done (Blaxhall and Daisley, 1973). One drop of hemolyzed blood was transferred onto Thoma lamella and examined under a light microscope (Soif, XZS-107B model) with a magnification of X400 (Blaxhall and Daisley, 1973). Leucocytes counting was done in blood samples after proper dilution into Turck solution (Blaxhall, 1981). Hemoglobin (HGB) concentration was determined according to the cyano-methemoglobin procedure (Kit 525- A; Sigma Chemical, St. Louis, MO, USA) (Blaxhall and Daisley, 1973). In this case 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 a hemoglobin standard (Azizoglu and Cengizler, 1996). Hematocrit was determined as in Wilhelm et al. (1992). Nonclotted blood was transferred to a microhematocrit capillary, afterward centrifuged at 14000 g for 5 min and read against a standard cart.

Statistical analysis

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SPSS (Statistical Package for Social Sciences) 9.0 for Windows statistic program was used for analyses of data. Biochemical and hematological data were analyzed using SPSS 9.0 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 and hematological parameters were shown in table 2 and table 3.

Table 2: Changes on the biochemical parameters in blood of carp with propolis, arsenic and arsenic+propolis groups

Biochemical parameters	Control	Propolis (10 mg/L)	Arsenic (0.01 mg/L)	Arsenic + Propolis (0.01 mg/L)+(10 mg/L)
Metabolites (mg dL^{-1})				
Albumin	**0.86± 0.13**	**0.88±0.10**	**0.91±0.10**	**0.92±0.12**
Globulin	**2.40 ± 0.21^{a}**	**2.35 ± 0.23^{a}**	**1.06 ± 0.22^{c}**	**1.97 ± 0.15^{b}**
Glucose	**128.0 ± 1.25^{b}**	**137.2 ± 1.79^{a}**	**75.00 ± 2.53^{d}**	**92.50 ± 1.70^{c}**
Creatinine	**0.20 ± 0.01**	**0.19 ± 0.02**	**0.23 ± 0.02**	**0.21 ± 0.02**
Total Protein	**3.38 ± 0.62**	**3.27 ± 0.54**	**2.38 ± 0.21**	**2.94 ± 0.33**
Triglycerides	**96,14 ± 2.20^{c}**	**99.17 ± 3.60^{c}**	**179.14 ± 5.70^{a}**	**139.10 ± 4.10^{b}**
Urea	**4.20 ± 0.40^{c}**	**4.70 ± 0.51^{c}**	**10.21 ± 1.20^{a}**	**7.25 ± 0.80^{b}**
Total cholesterol	**19.27 ± 1.81^{c}**	**24.13 ± 2.33^{c}**	**65.70 ± 3.86^{a}**	**34.70 ± 3.20^{b}**
Electrolyts (mmol L^{-1})				
Calcium	**14.79 ± 0.86^{a}**	**13.20 ± 0.90^{a}**	**9.12 ±0.25^{c}**	**10.44 ± 0.79^{b}**
Cobalt	**173.14 ± 2.60^{c}**	**175.77 ± 3.10^{c}**	**208.10 ± 3.11^{a}**	**191.10 ± 2.60^{b}**
Phosphate	**11.58 ± 0.84^{a}**	**11.70 ± 0.67^{a}**	**6.83 ± 0.42^{c}**	**9.42 ± 0.35^{b}**
Potassium	**1.86 ± 0.09^{a}**	**1.78 ± 0.10^{b}**	**1.04 ± 0.03^{d}**	**1.30 ± 0.06^{c}**
Sodium	**141.7 ± 3.80^{a}**	**141.5 ± 3.67^{a}**	**107.6 ± 7.60^{c}**	**128.70 ± 3.70^{b}**
Enzymes (IU L^{-1})				
ALP	**36.10 ± 1.17^{a}**	**35.80 ± 1.20^{a}**	**21.10 ± 1.80^{c}**	**28.75 ± 1.42^{b}**
ALT	**28.14 ± 2.03^{c}**	**27.79 ± 1.96^{c}**	**76.10 ± 2.10^{a}**	**42.14 ± 0.80^{b}**
Amylase	**93.14 ± 1.87^{a}**	**89.70 ± 2.59^{a}**	**27.18 ± 8.12^{c}**	**65.75 ± 4.17^{b}**
AST	**261.5 ± 3.76^{c}**	**265.7 ± 3.84^{c}**	**562.10 ± 5.90^{a}**	**357.10 ± 4.10^{b}**
LDH	**375.2 ± 6.80^{c}**	**392.1 ± 7.84^{c}**	**586.2 ± 5.60^{a}**	**476.8 ± 7.10^{b}**

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

Our results indicated that there were statistically significant decreases in the levels of glucose and globulin in the arsenic group (P<0.05). But levels of triglyceride, urea and total cholesterol in the arsenic group increased significantly in comparison to the control group. The arsenic+propolis treatment caused statistically significant increases in the levels of glucose and globulin compared with the arsenic group (P<0.05). Also, levels of triglyceride, urea and total cholesterol decreased by use of arsenic and propolis combination.

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Furthermore, there were no statistically significant changes in levels of albumin, creatinine, and total cholesterol in any of the groups (P>0.05). In addition there were significant increases in levels of cobalt, ALT, AST, LDH (P<0.05). But there were significant decreases in values of calcium phospate, sodium, ALP and amylase compared to the control group (Table 2). In arsenic+propolis group, there were decreases in values of cobalt, ALT, AST, LDH while there were significant increases in levels of calcium, phosphate, sodium, ALP and amylase compared to the arsenic group (P<0.05) (Table 2). Hematological parameters in treated propolis carp did not change compared with carp in the control group (P>0.05) (Table 3). But there were statistically significant decreases in levels of hematocrit, hemoglobin, erythrocyte counts and granulocyte counts in the exposed arsenic group compared to the control group (P<0.05). There were significant increases in levels of leucosyte, agranulocyte, MCV, MCH and MCHC in the arsenic group. The levels of leucocyte, agranulocyte, MCV, MCH and MCHC were significantly decreased by combination of arsenic and propolis (P<0.05) (Table 3).

Table 3: Changes on the hematologic parameters in blood of carp with propolis, arsenic and arsenic+propolis groups

Hematological parameters	Control	Propolis (10 mg/L)	Arsenic (0.01mg/L)	Arsenic+Propolis (0.01 mg/L+10 mg/L)
Leukocyte	**8.19 ± 0.48^{c}**	**8.42 ± 0.33^{c}**	**13.47 ± 0.32^{a}**	**10.66 ± 0.41^{b}**
Granulocytes (%)	**92.0 ± 0.11^{a}**	**89.00 ±1.41^{a}**	**59.50 ± 1.37^{c}**	**80.20 ± 2.60^{b}**
Agranulocytes (%)	**18.0 ± 0.12^{c}**	**11.00 ± 1.43^{c}**	**40.50 ± 1.35^{a}**	**19.80 ± 2.63^{b}**
ErythrocyteCount ($mm^3/10^6$)	**1.68 ± 0.04^{a}**	**1.67 ± 0.03^{a}**	**0.86 ± 0.05^{c}**	**1.26 ± 0.05^{b}**
Hemoglobin (g/dL)	**9.10 ± 0.10^{a}**	**9.33 ± 0.18^{a}**	**7.67 ± 0.87^{c}**	**8.25 ± 0.21^{b}**
Hematocrit (%)	**36.02 ± 0.91^{a}**	**36.2 ± 0.84^{a}**	**25.85 ± 0.60^{c}**	**32.60 ± 0.71^{b}**
Erythrocyte Indexes				
MCV (μ^3)	**214.40 ± 2.67^{c}**	**216.76 ± 2.89^{c}**	**304.11 ± 3.70^{a}**	**258.73 ± 3.11^{b}**
MCH (µg)	**54.16 ± 1.67^{c}**	**55.86 ± 1.74^{c}**	**90.23 ± 2.04^{a}**	**65.47 ± 1.92^{b}**
MCHC (%)	**25.26 ± 0.92^{b}**	**25.77 ± 0.81^{b}**	**29.67± 0.62^{a}**	**25.30 ± 0.71^{b}**

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

Discussion

In the present study, levels of leucocytes, agranulocytes, MCV, MCH and MCHC in the arsenic group increased, in contrast to decreased levels of erythrocyte count, granulocytes, hemoglobin and hematocrit. These values can be a marker of anemia with subsequent result of inhibition of erythropoesis in the hemopoietic system (Lavanya et al., 2011). Furthermore, the reason of increase in the MCV, MCH and MCHC values may be macrocytic type anemia. In addition, high leukocyte values depend on stress factors resulted in regulatory effects of toxic substances on the immune system (Das and Mukharjee, 2003; Dobsikova et al., 2006). There have

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been many studies on showing alike changes in blood parameters depending on various stress factors. For example, *Salmo gairdneri* exposed to lead indicated a decrease in blood parameters, such as erythrocyte number, hemoglobin level and hematocrit value (Johansson-Sjobeck and Larsson, 1978). It was also reported that there was an important decrease in the hematocrit value of *Salmo gairdneri* blood exposed to cadmium (Haux and Larsson, 1984). Also, exposure of *Oncorhynchus kisutsh* to zinc caused important decreases in hemoglobin and hematocrit values (McLeay, 1975). Our results are also in accordance with these previously reported results (Modi et al., 2006; Kalia et al., 2007; Lavanya et al., 2011). Arsenic is known to generate free radicals (Rana et al., 2010). It is well known that propolis is an antioxidant (Kanbur et al., 2009; Moreira et al., 2008). Levels of globulin, glucose, sodium, calcium, phosphate, amylase, ALP and granulocyte, erythrocyte, hemoglobin, hematocrit in arsenic+propolis group increased, whereas triglyceride, urea, total cholesterol, cobalt, ALT, AST, LDH and leucocyte, agranulocyte, MCV, MCH, MCHC levels reduced according to the arsenic group.

The toxic effects of arsenic on the inhibition of erythropoiesis in hematopoietic systems and the changes of biochemical and hematological parameters have been prevented by propolis. The elevated level of arsenic concentration in aquatic ecosystem affects various physiological systems such as growth, reproduction, ion regulation, immune function, enzyme activities and histopathology of fish (Pedlar et al., 2002; Data et al., 2009). Arsenic may cause oxidative stress in the liver of fish and bring about alterations in hematological parameters. Arsenic exposure may cause decreases in the counting of white and red blood cells (Kavitha et al., 2010). Hematological profiles of fish are widely used to demonstrate the environmental pollution in aquatic ecosystems (Carvalho and Fernandes, 2006). These parameters are also used to notice the physiological status of lives and indicators of stress (Adhikari et al., 2004; Lal Shah, 2010). Tripathi et al. showed that hemoglobin levels of *Clarias batrachus* exposed to arsenic decreased (Tripathi et al., 2003). Work on the elemination of toxic substance like arsenic from fish is very important for human health (Adhikari et al., 2004). Certain antioxidant agents can be used to eleminate and suppress the damages of toxic matters such as arsenic. The present study opens a new perspective on this investigation of biological properties of propolis with respect to the hematological and biochemical parameters in blood of carp. The propolis exists in natural environmental conditions and has biologically benefital effects on living organisms that live in natural areas.

Our study indicated that propolis can repair the deterioration caused by arsenic in fish which was demonstrated by analyses of hematological and biochemical parameters. The biological properties of propolis are mostly attributed to the phenolic components such as flavonoids (Mani et al., 2006). It has been reported that flavonoids have biological effects, including antibacterial, antiviral, anti-inflammatory, antiallergic, antioxidant and vasodilatory activities (Mani et al., 2006). Propolis treating to carp caused a protective effect against arsenic damage.

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We suggest that propolis be used as a protective in prevention of hematopoietic organ injuries and on other degenerative diseases in fish due to arsenic effects. In future, this work may shed light on investigations on biologic activities of new extracts of natural products such as propolis on aquatic organisms.

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