Vol. 108: 241–249, 2014
doi: 10.3354/dao02714

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published April 3

Antioxidant effects of propolis on carp *Cyprinus carpio* exposed to arsenic: biochemical and histopathologic findings

Zeliha Selamoglu Talas[1,*], Mehmet Fuat Gulhan[1], Kenan Erdogan[2], Ibrahim Orun[2]

[1]Department of Biology, Faculty of Arts and Science, Nigde University, Nigde 51200, Turkey
[2]Department of Biology, Faculty of Arts and Science, Aksaray University, Aksaray 68100, Turkey

ABSTRACT: Propolis, a resinous material produced by worker bees from the leaf buds and exudates of plants, is reported to possess various therapeutic properties. The aim of this study was to investigate the effects of propolis on biochemical parameters and histopathologic findings in carp *Cyprinus carpio* L. exposed to arsenic. A sublethal concentration of arsenic (0.01 mg l^{-1}) and/or 10 mg l^{-1} propolis were administered to fish for 1 wk. Catalase (CAT) activities and malondialdehyde (MDA) levels were determined in liver, gill and muscle tissues in control, arsenic only, propolis only and arsenic+propolis treatment groups. Results showed that CAT activity decreased in the arsenic group compared to the control and propolis groups. CAT activity in the arsenic+ propolis group was significantly higher compared to the arsenic group. MDA levels in fish exposed to 0.01 mg l^{-1} arsenic significantly increased compared to the control group. However, MDA levels in the arsenic+propolis group were significantly lower compared to the arsenic group. Histopathological changes in the liver, gill and muscle tissues of carp were examined by light microscopy: various changes were observed in all tissues of fish in the arsenic group. Propolis showed important antioxidant effects against arsenic toxicity in all fish tissues.

KEY WORDS: Oxidative stress · Heavy metal · Natural remedy · Histology · Fish

INTRODUCTION

Arsenic is the most widespread environmental contaminant, arising through natural phenomena such as weathering of geochemical sources and from anthropogenic activities such as mining, metal alloying and coal burning (Zhang et al. 2012). The accumulation of arsenic in organisms in contaminated water is an important environmental issue, because it may affect all members of a food web, including fish (Shah et al. 2009). In aquatic environments, arsenic exists as As(III) and As(V) (Kavitha et al. 2010). These types of arsenic can accumulate in many aquatic organisms and may catalyse the oxidation of arsenite to arsenate and promote the formation of methylarsines through a biomethylation reaction (Hughes 2002). Trivalent arsenic toxicity may occur either directly by connecting with sulfphydryl (–SH) groups in proteins while arsenate interferes with phosphorylation reactions, or indirectly by production of reactive oxygen species (ROS). Partial oxidative stress may occur with arsenic toxicity (Banerjee et al. 2009).

Oxidative stress has previously been analysed using a lipid peroxidation assay (Orun et al. 2008, Miranda et al. 2010). Increased levels of malondialdehyde (MDA), the last product of lipid peroxidation, are used as an indicator of local tissue damage (Duran & Talas 2009, Miranda et al. 2010, Gulhan et al. 2012). Lipid peroxides can change properties of biological membranes, resulting in heavy cell damage. In order to minimize such damage, cells have defence systems including both enzymatic (e.g. catalase) and nonenzymatic (e.g. flavonoid) processes.

*Corresponding author: ztalas@nigde.edu.tr

Histological changes in animal tissues provide a rapid method to detect effects of irritants in various tissues and organs. Liver, gill and muscle are considered the most important tissues, as toxic agents metabolized actively in organs such as the liver have the tendency to accumulate in fish (Roy & Bhattacharya 2006), and the gills play a key role as a conduit between the organism and its environment. Moreover, fish plays an important role in the diet of humans and is most often consumed in the form of fillets, i.e. muscle tissue.

External antioxidants and protective compounds must be consumed when toxic agents exceed normal levels. Identifying new antioxidants is an active field of biochemistry. In recent years, several organic forms of antioxidant molecules have been studied as possible natural therapeutic and preventive agents. Among these natural agents, researchers have become interested in propolis (Talas & Gulhan 2009, Talas et al. 2012, Gülçin et al. 2010, Beyraghdar Kashkooli et al. 2011, Gulhan et al. 2012). Propolis is a brownish resinous material collected by worker bees from the leaf buds of trees such as birch, poplar, pine, alder, willow and palm. Bees also use material exuded from wounds in plants (e.g. lipophilic material on leaves, mucilages, gums, resins, lattices) in order to manufacture propolis. Once collected, this material is enriched with salivary and enzymatic secretions. It is then used by bees to cover hive walls, fill cracks or gaps and embalm invading insects that have been killed in the hive (Castaldo & Capasso 2002). Globally, propolis is a popular agent for use in traditional medicine and as a food supplement to benefit human health (Gülçin et al. 2010). Propolis possesses various biological properties such as antibacterial, antifungal, antiviral, anti-inflammatory, anticancer and immunomodulatory activities (Banskota et al. 2000). Biological actions of propolis are generally attributed to its constituents of plant origin, mainly phenolics (Burdock 1998). Flavonoids are well known to possess antioxidant effects via their free-radical-scavenging and metal-chelating properties (Rice-Evans 2001). Natural antioxidants are essential for homeostasis in biological systems, including both fish and humans. Due to its antioxidant and preservative effects, propolis may both prolong the physiological functions of some aquatic organisms and contribute to the health benefits of consumers of aquatic animals (Talas & Gulhan 2009, Talas et al. 2012, Gulhan et al. 2012).

In this study, we evaluated histological changes and biochemical parameters to understand the antioxidative effects of propolis on carp *Cyprinus carpio* exposed to arsenic.

MATERIALS AND METHODS

Preparation of propolis extract solution

The chemical composition of propolis is very complex. Over 300 components have been identified to date, and its composition is variable, depending upon the local flora. In our study, propolis was collected from a farm in the village of Kocaavsar in Balikesir, Turkey. Propolis was dissolved to 30% in ethanol, protected from light and moderately shaken for 1 d at room temperature. The extract was then filtered twice, dried and stored in sealed bottles at 4°C until use (Mani et al. 2006).

Experimental design

Carp were obtained from Azatli Dam Lake in Nigde, Turkey. Fish were acclimatized for 15 d in a 200 l tank. Airflow in the tank was continuously provided and fish were given artificial dry food once daily. Physical and chemical properties of tank water are provided in Table 1.

After acclimatization, fish were randomly assigned to one of 4 experimental treatment groups, each consisting of 28 animals (4 replicate tanks per treatment, each tank containing 7 fish). The average weight of the fish was between 500 and 600 g.

Fish in the first group were used as controls and were given no treatment. Propolis at an antioxidant concentration of 10 mg l^{-1} (Talas & Gulhan 2009), was administered to the tank water for the second group for 1 wk, and the fish were not fed for 12 h before the application. Arsenic (As_2O_3) (98% pure; Aldrich) at 0.01 mg l^{-1} (Schlenk et al. 1997) was added to the third group of tanks for 1 wk, and they were not fed for 12 h before. Both 10 mg l^{-1} propolis and 0.01 mg

Table 1. Parameters (mean ± SD) of the water used in the experiment

Parameter (mg l^{-1})	Before treatment	After treatment
Dissolved oxygen	7.7 ± 0.2	7.6 ± 0.5
Chemical oxygen demand	15.4 ± 0.6	16.8 ± 0.4
Suspended solids	34.8 ± 1.2	41.4 ± 1.2
Calcium	122.0 ± 1.5	110.1 ± 1.4
Sodium	21.7 ± 0.8	17.3 ± 0.7
Chloride	15.0 ±1.5	17.0 ±1.6
Total nitrogen	5.3 ± 0.2	6.2 ± 0.3
Hardness ($CaCO_3$)	170.3 ± 4.1	167.2 ± 1.9
Temperature (°C)	18.9 ± 0.7	21 ± 0.3
pH	7.6 ± 0.3	7.7 ± 0.2

l^{-1} arsenic (As_2O_3) were added to the last group of tanks for 1 wk; these fish were also not fed for 12 h beforehand (Talas et al. 2012). At the end of the treatment period, we randomly sampled 2 fish from each tank for assessment of biochemical parameters; thus 8 fish (4 replicates) were sampled per treatment.

The experiments were performed in accordance with the guidelines for fish research from the National Institute of Health and approved by the Ethical Committee of the Science Institute at Nigde University, Nigde, Turkey.

Preparation of tissues for biochemical analyses

After the treatments, fish were anaesthetised with clove oil. Tissues of fish were removed, frozen in liquid nitrogen and stored at −80°C until used. The tissues were separated into 2 parts for determination of total protein level, catalase (CAT) activity and lipid peroxidation. Tissues were weighed and then homogenized in 100 ml of 2 mM phosphate buffer, pH 7.4, using a PCV Kinematica Status Homogenizer. Homogenized samples were sonicated for 1.5 min (30 s sonications interrupted by 30 s pauses, on ice). Samples were then centrifuged at 12 000 × *g* (15 min, 4°C), and the supernatants, if not used immediately for the enzyme assays, were kept frozen at −80°C. Supernatants were used for the determination of total protein and CAT activity. The second part of the tissue homogenate was used for lipid peroxidation analysis. Tissues were washed 3 times with ice-cold 0.9% NaCl solution and homogenized in 1.15% KCl. The homogenates were assayed for malondialdehyde (MDA) levels (Orun et al. 2005).

Total protein assay

Supernatants of tissues were used to determine total protein levels. The total protein level was quantified by the colorimetric method of Lowry et al. (1951) using BSA as the standard.

Determination of MDA levels and CAT activity

Lipid peroxidation in the tissues of carp was measured according to the concentration of thiobarbituric acid (TBA) reactive substances. The amount of MDA was used as an index of lipid peroxidation (Yagi 1984). In the TBA test reaction, MDA and TBA react with the production of pink pigment with a maximum absorption at 532 nm. The reaction was performed at pH 2 to 3 at 90°C for 15 min. The samples were mixed with 2 volumes of cold 10% (w/v) trichloroacetic acid for protein precipitation. The precipitate was pelleted by centrifugation, and an aliquot of the supernatant was reacted with an equal volume of 0.67% (w/v) TBA in a boiling water bath for 10 min. After cooling, absorbance was read at 532 nm. The results are expressed as nmol g^{-1} wet tissue.

CAT activities in the tissues were determined by measuring the composition of hydrogen peroxide at 240 nm, according to the method of Aebi (1984), and are expressed as kU g^{-1} protein, where kU is the first-order rate constant.

Preparation of tissue samples for histological measurement

After each exposure, both the experimental and control fish were sacrificed for histopathological examination. Liver, gill and muscle tissues were transferred to 10% formaldehyde solution for 24 h for fixation. Following fixation, the tissues were washed for 24 h with running tap water in a beaker. The tissues were dehydrated in graded ethyl alcohol solutions. Tissues were cleared twice in xylene and embedded in paraffin. Sections (5–10 µm thick) were made and stained with haematoxylin and eosin, and all sections were examined under light microscopy. We haphazardly selected 3 different fields of view per slide per fish, and the results from each observation were combined for the final results.

Statistical analysis

SPSS 16.0 for Windows was used to analyse the data. Differences between biochemical parameter means were determined using Duncan's multiple range test in which the significance level was defined as $p < 0.05$. Data are given as means ± SD ($n = 8$).

RESULTS

Biochemical assay

Changes in biochemical parameters, viz. CAT activities and MDA levels, in liver, gill and muscle tissues of carp exposed to 0.01 mg l^{-1} arsenic and 10 mg l^{-1}

Table 2. *Cyprinus carpio*. Changes in mean ± SD (n = 8) catalase activities (kU g^{-1}) of liver, gill and muscle tissues of carp in control, propolis only, arsenic only and arsenic+ propolis groups. Within rows, values with different superscripts are significantly different (Duncan's multiple range test, $p < 0.05$)

Tissue	Control	Propolis	Arsenic	Arsenic+ propolis
Liver	45.53 ± 1.45^{a}	44.11 ± 1.12^{a}	21.49 ± 1.22^{c}	37.83 ± 0.78^{b}
Gill	1.59 ± 0.08^{a}	1.56 ± 0.06^{a}	0.87 ± 0.02^{c}	1.26 ± 0.04^{b}
Muscle	2.06 ± 0.12^{a}	1.96 ± 0.14^{a}	0.80 ± 0.11^{c}	1.46 ± 0.13^{b}

Table 3. *Cyprinus carpio*. Changes in mean ± SD (n = 8) malondialdehyde levels (nmol g^{-1}) of liver, gill and muscle tissues of carp in control, propolis only, arsenic only and arsenic+propolis groups. Within rows, values with different superscripts are significantly different (Duncan's multiple range test, $p < 0.05$)

Tissue	Control	Propolis	Arsenic	Arsenic+ propolis
Liver	0.18 ± 0.02^{c}	0.19 ± 0.03^{c}	0.54 ± 0.05^{a}	0.24 ± 0.02^{b}
Gill	1.63 ± 0.13^{c}	1.79 ± 0.21^{c}	2.89 ± 0.23^{a}	2.14 ± 0.18^{b}
Muscle	1.13 ± 0.09^{c}	1.25 ± 0.10^{c}	3.29 ± 0.27^{a}	1.85 ± 0.12^{b}

propolis are shown in Tables 2 & 3. CAT activities of liver, gill and muscle tissues of carp in the propolis group did not change compared to the control group ($p > 0.05$; Table 2). CAT activities in liver, gill and muscle tissues of the arsenic group significantly decreased ($p < 0.05$) compared to the control group. We found significant improvements in CAT activities of all tissues in the arsenic+propolis group compared to the arsenic group (Table 2).

MDA is an important indicator for oxidative damage, and MDA levels of liver, gill and muscle tissues in the propolis group did not change compared to the control group ($p > 0.05$; Table 3). Compared to the control group, significant increases ($p < 0.05$) in MDA levels of liver, gill and muscle tissues were observed in fish exposed to arsenic, but we found that MDA levels of tissues were lower in the arsenic+propolis group compared to the arsenic group ($p < 0.05$; Table 3). According to the biochemical data, propolis may contribute to the antioxidative defence system of carp at a concentration of 10 mg l^{-1}. This tolerance can be explained by the antioxidant effects of propolis.

Histology

Liver. Hepatocytes are located among the sinusoids, forming cord-like structures known as hepatic cell cords. Hepatocytes have a roundish polygonal cell body containing a clear spherical nucleus typically with 1 nucleolus. No histopathological changes were observed in livers of the control fish (Fig. 1a). The histological changes noted in the propolis group, including infiltration of mononuclear cells, are shown in Fig. 1b. In the arsenic group, we observed infiltration of mononuclear cells, hydropic degeneration and congestion in the liver tissues (Fig. 1c). In the arsenic+ propolis group, significant changes in the liver tissues were observed. We found important differences in the

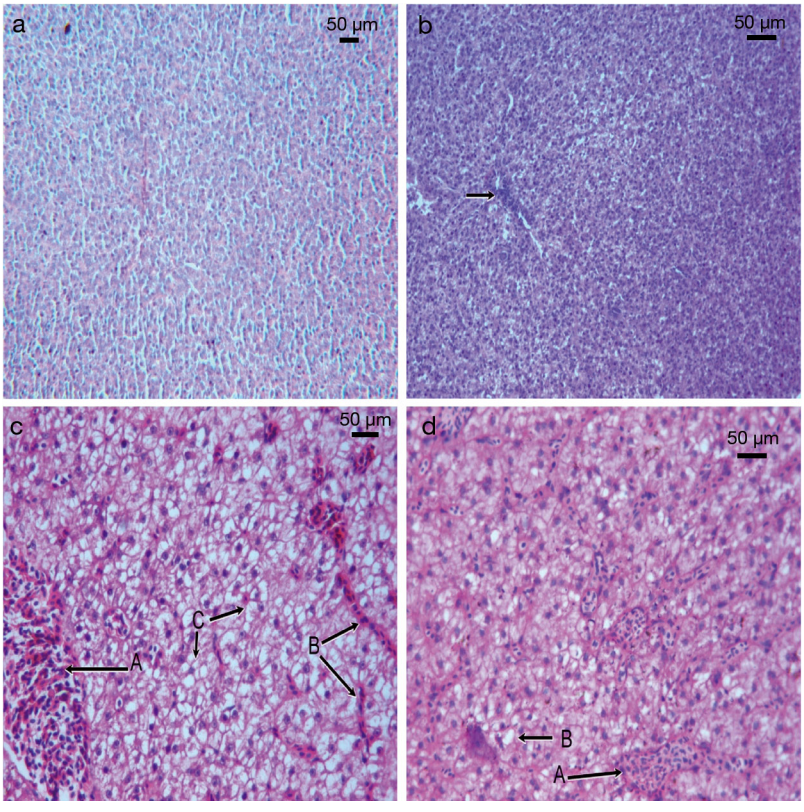

Fig. 1. *Cyprinus carpio*. Liver structure of (a) control fish; (b) fish treated only with propolis (arrow shows infiltration of mononuclear cells); (c) fish treated only with arsenic, showing congestion (A), hydropic degeneration (B) and infiltration of mononuclear cells (C); and (d) fish treated with arsenic+propolis, showing congestion (A) and hydropic degeneration (B). All images: H&E stain

histopathology of the liver between this group and the group that was treated with only arsenic. Congestion and hydropic degeneration were noticed in the arsenic+propolis group (Fig. 1d).

Gill. Gills comprise primary lamellae, with secondary lamellae found on the lateral sides of the primary lamellae. The surface of the gill lamellae is covered with simple squamous epithelial cells, and capillaries separated by pillar cells run parallel along the surface. The primary lamellae are lined by a thick stratified epithelium between the secondary lamellae. This region contains the mucous cells and chloride cells. No histopathological changes were observed in the gill of the control fish (Fig. 2a). The most common change in the propolis group was vacuolization (Fig. 2b). Histopathological results indicated that the gill was the primary target tissue affected by arsenic: vacuolization, necrosis (telangiectasias) adjacent to the secondary lamellae, cartilage damage, and fusion and shortening of the secondary lamellae were noted in the arsenic group (Fig. 2c). In the arsenic+propolis group, we observed important differences in the histopathology of the gill as compared to the arsenic group, including shortening and thickening of the secondary lamellae (Fig. 2d).

Muscle. No histopathological changes were observed in the muscle of control fish (Fig. 3a). Nuclear proliferation was apparent in muscle tissue of the propolis group (Fig. 3b), as well as in the muscle tissues of the arsenic group (Fig. 3c). In the arsenic+propolis group, we observed greater nuclear proliferation compared to the arsenic group (Fig. 3d).

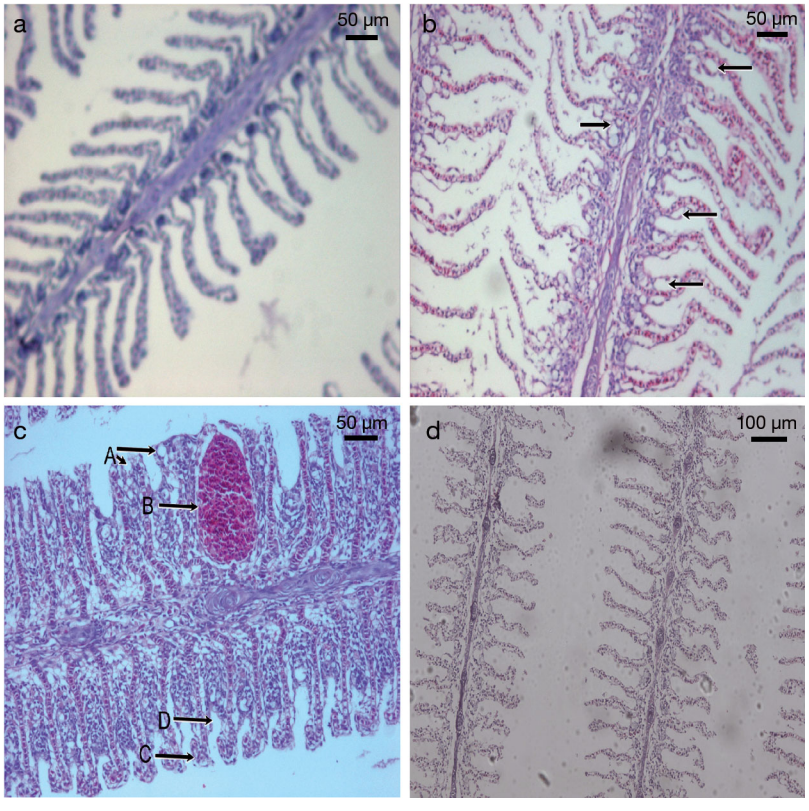

Fig. 2. *Cyprinus carpio*. Gill structure of (a) control fish; (b) fish treated only with propolis (arrows show vacuolization); (c) fish treated only with arsenic, showing vacuolization (A), necrosis (teleangiectasias) (B), a combination of secondary lamellae and cartilage damage (C) and fusion and shortening of the secondary lamellae (D); and (d) fish treated with arsenic+propolis, showing shortening and thickening of the secondary lamellae. All images: H&E stain

DISCUSSION

Organs of fish were selected on the basis of functional criteria that made them preferential targets, i.e. for heavy metal metabolism (liver), heavy metal uptake (gill) and effects of arsenic on the quality of fish fillets (muscle). Production of free radicals, which can be caused by arsenic, plays an important role in damage and loss of function in tissues and organs (Garg et al. 2009, Ventura-Lima et al. 2009, Talas et al. 2012). Lipid peroxidation, which results from the oxidative injury of saturated and unsaturated lipids, has been broadly used as a marker of the induction of oxidative damage in fish suffering from environmental stress induced by heavy metals (Gioda et al. 2007). In our study, MDA levels increased in all of the studied tissues after exposure to arsenic. These findings strongly suggest that arsenic caused generalized oxidative stress in the fish. Thus, the elevated production of ROS may be due to arsenic toxicity, although other effects, such as enzymatic inhibition or genotoxic damage, may also occur.

In recent years, ecotoxicological studies have investigated free radical damage and oxidative stress in different fish exposed to toxic metals and organic pollutants (e.g. cadmium, mercury, copper, arochlor and contaminated sediments; Orun et al. 2008, 2011, Gulhan et al. 2012, Talas et al. 2102). Extensive tissue lipid peroxidation caused increased MDA levels in liver, gill and muscle tissues (Berntssen et al. 2003, Orun et al. 2008, 2011).

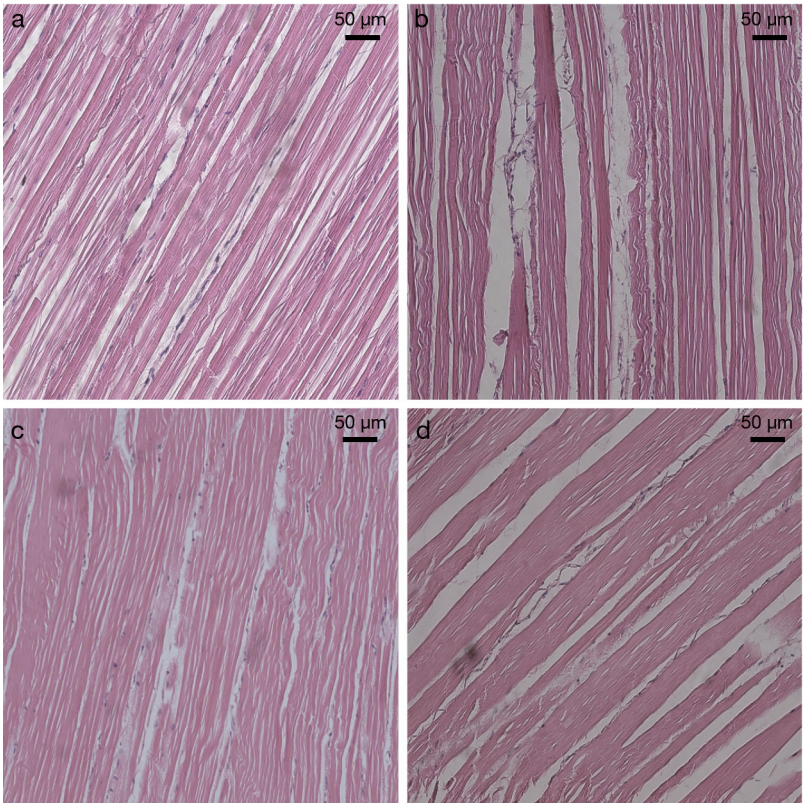

Fig. 3. *Cyprinus carpio*. Muscle structure of (a) control fish; (b) fish treated only with propolis; (c) fish treated only with arsenic; and (d) fish treated with arsenic+propolis. Images in panels b, c, and d show nuclear proliferation. All images: H&E stain

Increased MDA levels in aquatic organisms exposed to heavy metals have been demonstrated in some studies (Ates et al. 2008, Orun et al. 2008, 2011, Pandey et al. 2008, Ruas et al. 2008). As a marker of oxidative stress, MDA levels of liver, gill and muscle in our study increased in fish exposed to arsenic, but these increases were much less marked in tissues of fish in the arsenic+propolis group. In this case, oxidative stress was reduced with the application of propolis. These results provide direct evidence for the protective role of propolis, which confers therapeutic properties on the antioxidative defence system against heavy metals. According to biochemical data, a propolis concentration of 10 mg l^{-1} may contribute to the antioxidative defence system of carp.

The first line of defense against damaging effects of ROS is antioxidant enzymes such as CAT. Superoxide dismutase catalyses the dismutation of $O_2^{\cdot-}$ to H_2O_2, and CAT reduces H_2O_2 to $2H_2O$ (Atli et al. 2006). Van der Oost et al. (2003) investigated the impact of heavy metals (Cd^{2+}, Cr^{3+}) in an environmental survey and found that antioxidant enzyme activities decreased in fish exposed to heavy metals. Atli et al. (2006) reported that metals (Cd^{2+}, Cu^{2+}, Cr^{2+} and Zn^{2+}) stimulated CAT activity in the liver of Nile tilapia *Oreochromis niloticus* and that CAT activity was inhibited by Ag^{+} and Cd^{2+} *in vivo*. The inhibition of CAT activity was related to the binding of metal ions to –SH groups of the enzyme, increased hydrogen peroxide and/or superoxide radicals. CAT is the primary enzyme in scavenging H_2O_2, so more H_2O_2 is available for the production of hydroxide free radicals when CAT activity is inhibited (Atli et al. 2006).

In our study, CAT activities of liver, gill and muscle decreased with heavy metal exposure in the fish. The oxidative changes in various biochemical parameters of fish exposed to some toxic agents and heavy metals such as Cd^{2+} and Cr^{3+} have been reported (Talas et al. 2008, Li et al. 2010a,b, 2011). Similar findings have also been reported by other researchers (Orun et al. 2008, 2011, Ventura-Lima et al. 2009, Oliva et al. 2012).

Some natural therapeutic and preventive agents are available to defend against arsenic damage. Propolis is one of these natural therapeutic bee products (which also include pollen and royal jelly). These natural antioxidants are essential for homeostasis in many biological systems both in fish and humans. Increasing CAT activity after propolis application to fish exposed to arsenic may be related to the antioxidant effects of propolis. Our results are in accordance with earlier studies (Ramanathan et al. 2003, Nandi et al. 2008).

Histological findings are important in supporting biochemical assays. Histological changes in animal tissues provide a rapid method to detect effects of irritants, especially chronic effects, in various tissues and organs (Velmurugan et al. 2007). Liver tissue of fish is considered a major site of storage, biotransformation and excretion of heavy metals such as arsenic, and alterations in liver function are often indicative of pollution. The liver suffers from serious morphological alterations in fish exposed to heavy metals and is the main organ for detoxification (Ventura-Lima et al. 2009). Alterations in the liver may thus be useful as markers that indicate prior exposure to environmental stressors.

In our study, the infiltration of mononuclear cells, hydrophic degeneration and congestion were observed in the livers of fish exposed to arsenic. We found important histopathological differences in the livers of fish in the arsenic+propolis group compared to the group that was treated only with arsenic. Our results are also in accordance with previous reports (Pedlar et al. 2002, Roy & Bhattacharya 2006). Congestion and hydrophic degeneration were observed in liver tissues of the arsenic+propolis group. These analyses show that the damage in liver tissue of the propolis group was less severe relative to the arsenic group.

Necrosis and desquamation of secondary lamellar epithelium, intraepithelial oedema, fusion of adjacent secondary lamellae, haemorrhage at the primary lamellae, hypertrophy and hyperplasia of epithelial cells have been shown in experimental investigations (Erkmen et al. 2000, Cengiz & Unlu 2003). Erkmen et al. (2000) reported lifting of the epithelial layer from the gill lamellae, necrosis and degeneration of the secondary lamellae, oedema, shortening of the secondary lamellae, and club-shaped lamellae in the gills of guppies *Lebistes reticulatus* (= *Poecilia reticulata*) exposed to cyphenothrin. Shorter gill lamellae, fusion, complete destruction of the lamellae, increased vacuolation and an irregular appearance of gill lamellae were observed in *P. reticulata* exposed to chlorpyrifos (De Silva & Samayawardhena 2002). Our results thus corroborate results of previous studies (Pandey et al. 2008, Monteiro et al. 2010).

We observed vacuolization, necrosis adjacent to the secondary lamellae, cartilage damage and fusion and shortening of the secondary lamellae in gills of carp exposed to arsenic. In the arsenic+propolis group, histopatological changes were evident in the gills, including shortening and thickening of the secondary lamellae, compared to carp exposed to only arsenic. These changes in gill tissues were due to the therapeutic effects of propolis.

Fish plays an important role in the human diet. Fish fillets may be affected by heavy metals, such as arsenic, which can cause structural damage. We observed only nuclear proliferations in the muscle tissues of both the arsenic group and the arsenic+propolis group. Several studies have investigated the effects of heavy metals in fish muscle tissues (e.g. Palaniappan & Vijayasundaram 2008, Garg et al. 2009). The results of these studies are in line with our data. Histopathological studies are useful to evaluate the potential effects of accumulation of arsenic in fish. Our study showed that histopathological changes may originate from the toxic effects of arsenic. Identifying pathological alterations in organs following contact with toxicants is useful as a biomarker of exposure to and effects of pollutants. All of our histopathological observations indicated that exposure to sublethal concentrations of arsenic caused destructive effects in the gill, liver and muscle tissues of *Cyprinus carpio*.

Experimental studies on the elimination of toxic substances such as arsenic in fish are also very important for human health. Certain antioxidant agents can be used to eliminate and suppress the damage caused by toxic substances. Our study indicated that propolis can repair the deterioration caused by arsenic in fish, as revealed by our analyses of biochemical parameters and the histological assay. The therapeutic properties of propolis are mostly attributed to phenolic components such as flavonoids. Flavonoids have beneficial biological effects, including antibacterial, antiviral, anti-inflammatory, antiallergic, antioxidant and vasodilatory activities. Propolis caused a protective effect against arsenic damage in carp. We suggest that propolis can be used for protection of tissues and organs against degenerative diseases in fish caused by the accumulation of arsenic. Numerous chemical components of propolis have been reported, and this variety is primarily influenced by the botanical and geographical origins of propolis. More than 300 compounds including volatile organic compounds, flavonoid aglycones, phenolic acids and their esters, phenolic aldehydes, alcohols and ketones, sesquiterpenes, quinones, coumarins, steroids and amino acids have been reported among the components of propolis (Nakamura et al. 2010, Beyraghdar Kashkooli et al. 2011). The most common compounds found in propolis include phenolics, which play an important protective role against oxidative stress caused by toxic agents such as xenobiotics and heavy metals. Flavones, coumarins and many other phenolic compounds also have antioxidant activities as metal chelators and donors of hydrogen (Marcucci et al. 2000). All of these compounds are responsible for the biological and pharmacological activities of propolis.

In the future, this work may shed light on investigations of biological activities of new extracts from natural products such as propolis in aquatic organisms.

LITERATURE CITED

Aebi H (1984) Catalase *in vitro*. Methods Enzymol 105: 121–126

Ates B, Orun I, Talas ZS, Durmaz G, Yilmaz I (2008) Effects of sodium selenite on some biochemical and hematologi-

cal parameters of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) exposed to Pb^{2+} and Cu^{2+}. Fish Physiol Biochem 34:53–59

Atli G, Alptekin O, Tükel S, Canli M (2006) Response of catalase activity to Ag^{2+}, Cd^{2+}, Cr^{2+}, Cu^{2+} and Zn^{2+} in five tissues of freshwater fish *Oreochromis niloticus*. Comp Biochem Physiol C Toxicol Pharmacol 143:218–224

Banerjee P, Bhattacharyya SS, Bhattacharjee N, Pathak S, Boujedaini N, Belon P, Khuda-Bukhsh AR (2009) Ascorbic acid combats arsenic-induced oxidative stress in mice liver. Ecotoxicol Environ Saf 72:639–649

Banskota AH, Tezuka Y, Midorikawa K, Matsushige K, Kadota S (2000) Two novel cytotoxic benzofuran derivatives from Brazilian propolis. J Nat Prod 63:1277–1279

Berntssen MH, Aatland A, Handy RD (2003) Chronic dietary mercury exposure causes oxidative stress, brain lesions, and altered behaviour in Atlantic salmon (*Salmo salar*) parr. Aquat Toxicol 65:55–72

Beyraghdar Kashkooli O, Ebrahimi Dorcheh E, Mahboobi-Soofiani N, Samie A (2011) Long-term effects of propolis on serum biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). Ecotoxicol Environ Saf 74: 315–318

Burdock GA (1998) Review of the biological properties and toxicity of bee propolis (propolis). Food Chem Toxicol 36: 347–363

Castaldo S, Capasso F (2002) Propolis, an old remedy used in modern medicine. Fitoterapia 73:S1–S6

Cengiz EI, Unlu E (2003) Histopathology of gills in mosquitofish, *Gambusia affinis* after long-term exposure to sub lethal concentrations of malathion. J Environ Sci Health B 38:581–589

De Silva PM, Samayawardhena LA (2002) Low concentrations of lorsban in water result in far reaching behavioral and histological effects in early life stages in guppy. Ecotoxicol Environ Saf 53:248–254

Duran A, Talas ZS (2009) Biochemical changes and sensory assessment on tissues of carp (*Cyprinus carpio*, Linnaeus 1758) during sale conditions. Fish Physiol Biochem 35: 709–714

Erkmen B, Caliskan M, Yerli SV (2000) Histopathological effects of cyphenothrin on the gills of *Lebistes reticulatus*. Vet Hum Toxicol 42:5–7

Garg S, Gupta RK, Jain KL (2009) Sublethal effects of heavy metals on biochemical composition and their recovery in Indian major carps. J Hazard Mater 163:1369–1384

Gioda CR, Lissner LA, Pretto A, da Rocha JB and others (2007) Exposure to sublethal concentrations of Zn(II) and Cu(II) changes biochemical parameters in *Leporinus obtusidens*. Chemosphere 69:170–175

Gülçin I, Bursal E, Sehitoglu MH, Bilsel M, Goren AC (2010) Polyphenol contents and antioxidant activity of lyophilized aqueous extract of propolis from Erzurum, Turkey. Food Chem Toxicol 48:2227–2238

Gulhan MF, Duran A, Talas ZS, Kakoolaki S, Mansouri SM (2012) Effects of propolis on microbiologic and biochemical parameters of rainbow trout (*Oncorhynchus mykiss*) after exposure to the pesticide. Iran J Fish Sci 11:490–503

Hughes MF (2002) Arsenic toxicity and potential mechanisms of action. Toxicol Lett 133:1–16

Kavitha C, Malarvizhi A, Kumaran S, Ramesh M (2010) Toxicological effects of arsenate exposure on hematological, biochemical and liver transaminases activity in an Indian major carp, *Catla catla*. Food Chem Toxicol 48: 2848–2854

Li ZH, Zlabek V, Grabic R, Li P, Machova J, Velisek J, Randak T (2010a) Effects of exposure to sublethal propiconazole on intestine-related biochemical responses in rainbow trout, *Oncorhynchus mykiss*. Chem Biol Interact 185:241–246

Li ZH, Li P, Randak T (2010b) Ecotoxocological effects of short-term exposure to a human pharmaceutical Verapamil in juvenile rainbow trout (*Oncorhynchus mykiss*). Comp Biochem Physiol C Toxicol Pharmacol 152:385–391

Li ZH, Zlabek V, Velisek J, Grabic R and others (2011) Acute toxicity of carbamazepine to juvenile rainbow trout (*Oncorhynchus mykiss*): effects on antioxidant responses, hematological parameters and hepatic EROD. Ecotoxicol Environ Saf 74:319–327

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurements with the folin phenol reagent. J Biol Chem 193:265–275

Mani F, Damasceno HCR, Novelli ELB, Martins EAM, Sforcin JM (2006) Propolis: effect of different concentrations, extracts and intake period on seric biochemical variables. J Ethnopharmacol 105:95–98

Marcucci MC, Ferreres F, Custodio AR, Ferreira MMC, Bankova VS, Garcia-Viguera C, Bretz WA (2000) Evaluation of phenolic compounds in Brazilian propolis from different geographic regions. Z Naturforsch C 55:76–81

Miranda LE, Capellini VK, Reis GS, Celotto AC, Carlotti CG, Evora PR (2010) Effects of partial liver ischemia followed by global liver reperfusion on the remote tissue expression of nitric oxide synthase: lungs and kidneys. Transplant Proc 42:1557–1562

Monteiro DA, Rantin FT, Kalinin AL (2010) Inorganic mercury exposure: toxicological effects, oxidative stress biomarkers and bioaccumulation in the tropical freshwater fish matrinxa, *Brycon amazonicus* (Spix and Agassiz, 1829). Ecotoxicology 19:105–123

Nakamura R, Nakamura R, Watanabe K, Oka K, Ohta S, Mishima S, Teshima R (2010) Effects of propolis from different areas on mast cell degranulation and identification of the effective components in propolis. Int Immunopharmacol 10:1107–1112

Nandi D, Patra RC, Ranjan R, Swarup D (2008) Role of co-administration of antioxidants in prevention of oxidative injury following sub-chronic exposure to arsenic in rats. Vet Arh 78:113–121

Oliva M, Vicente JJ, Gravato C, Guilhermino L, Galindo-Riano MD (2012) Oxidative stress biomarkers in Senegal sole, *Solea senegalensis*, to assess the impact of heavy metal pollution in a Huelva estuary (SW Spain): seasonal and spatial variation. Ecotoxicol Environ Saf 75:151–162

Orun I, Ates B, Selamoglu Z, Yazlak H, Ozturk E, Yilmaz I (2005) Effects of various sodium selenite concentrations on some biochemical and hematological parameters of rainbow trout (*Oncorhynchus mykiss*). Fresenius Environ Bull 14:18–22

Orun I, Talas ZS, Ozdemir I, Alkan A, Erdogan K (2008) Antioxidative role of selenium on some tissues of (Cd^{2+}, Cr^{3+})-induced rainbow trout. Ecotoxicol Environ Saf 71: 71–75

Orun I, Talas ZS, Alkan A (2011) Modulating effect of selenium on gills of fish exposed to heavy metals. Fresenius Environ Bull 20:104–108

Palaniappan PR, Vijayasundaram V (2008) Fourier transform infrared study of protein secondary structural changes in the muscle of *Labeo rohita* due to arsenic intoxication. Food Chem Toxicol 46:3534–3539

Pandey S, Parvez S, Ansari RA, Ali M and others (2008) Effects of exposure to multiple trace metals on biochemical, histological and ultrastructural features of gills of a freshwater fish, *Channa punctata* Bloch. Chem Biol Interact 174:183–192

Pedlar RM, Ptashynski MD, Evans R, Klaverkamp JF (2002) Toxicological effects of dietary arsenic exposure in lake whitefish (*Coregonus clupeaformis*). Aquat Toxicol 57: 167–189

Ramanathan K, Shila S, Kumaran S, Panneerselvam C (2003) Protective role of ascorbic acid and a-tocopherol on arsenic induced microsomal dysfunction. Hum Exp Toxicol 22:129–136

Rice-Evans C (2001) Flavonoid antioxidants. Curr Med Chem 8:797–807

Roy S, Bhattacharya S (2006) Arsenic-induced histopathology and synthesis of stress proteins in liver of *Channa punctatus*. Ecotoxicol Environ Saf 65:218–229

Ruas CB, Carvalho C, Arauj HS, Espindola EL, Fernandes MN (2008) Oxidative stress biomarkers of exposure in the blood of three cichlid species from a polluted river. Ecotoxicol Environ Saf 71:86–93

Schlenk D, Colley WC, El-Alfy A, Kirby R, Griffin BR (1997) Effect of arsenite, arsenate, and the herbicide monosodium methyl arsonate (MSMA) on hepatic metallothionein expression and lipid peroxidation in channel catfish. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 118:177–183

Shah AQ, Kazi TG, Arain MB, Jamali MK and others (2009) Accumulation of arsenic in different fresh water fish species — potential contribution to high arsenic intakes. Food Chem 112:520–524

Talas ZS, Gulhan MF (2009) Effects of various propolis concentrations on biochemical and hematological parameters of rainbow trout (*Oncorhynchus mykiss*). Ecotoxicol Environ Saf 72:1994–1998

Talas ZS, Orun I, Ozdemir I, Erdogan K, Alkan A, Yilmaz I (2008) Antioxidative role of selenium against the toxic effect of heavy metals (Cd^{2+}, Cr^{3+}) on liver of rainbow trout (*Oncorhynchus mykiss* Walbaum 1792). Fish Physiol Biochem 34:217–222

Talas ZS, Dundar SP, Gulhan MF, Orun I, Kakoolaki S (2012) Effects of propolis on some blood parameters and enzymes in carp exposed to arsenic. Iran J Fish Sci 11: 405–414

van der Oost R, Beyer J, Vermeulen NPE (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Pharmacol 13: 57–149

Velmurugan B, Selvanayagam M, Cengiz EI, Unlu E (2007) Histopathology of lambda-cyhalothrin on tissues (gill, kidney, liver and intestine) of *Cirrhinus mrigala*. Environ Toxicol Pharmacol 24:286–291

Ventura-Lima J, Castro MR, Acosta D, Fattorini D and others (2009) Effects of arsenic (As) exposure on the antioxidant status of gills of the zebrafish *Danio rerio* (Cyprinidae). Comp Biochem Physiol C Toxicol Pharmacol 149: 538–543

Yagi K (1984) Assay of lipid peroxidation in blood plasma or serum. Methods Enzymol 105:328–331

Zhang W, Huang L, Wang WX (2012) Biotransformation and detoxification of inorganic arsenic in a marine juvenile fish *Terapon jarbua* after waterborne and dietborne exposure. J Hazard Mater 221/222:162–169

Editorial responsibility: Thomas Braunbeck, Heidelberg, Germany

Submitted: November 18, 2013; Accepted: December 23, 2013
Proofs received from author(s): March 12, 2014