The effects of propolis on gill, liver, muscle tissues of rainbow trout *(Oncorhynchus mykiss)* exposed to various concentrations of cypermethrin

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Received: December 2013 Accepted: June 2014

Abstract

The aim of this study was to investigate the therapeutic effects of propolis for the toxicity of cypermethrin (CYP) on histopathological changes in tissues of rainbow trout (*Oncorhynchus mykiss*). CYP is one of the most toxic pyrethroids highly for the aquatic organisms. The fish were exposed to three sublethal concentrations of CYP (0.0041, 0.0082 and 0.0123 ppm). In addition, different concentrations of propolis (10, 20 and 30 ppm) were used in the investigation for the period of 96 h. Propolis was collected by honeybees from different plants to prevent oxidative damages as an antioxidant. The therapeutic concentration of propolis was determined at 10 ppm. 10 ppm propolis was added to three cypermethrin concentrations. Shorting the secondary lamellae , fusion of secondary lamellae, oedema, necrosis, vacuolization and cartilage damage in gill tissue of fish exposed to CYP were observed by histopatological analyses. Hepatic lesions in liver tissue of fish exposed to CYP were characterized as hydropic degeneration, necrosis, mononuclear cell infiltration and narrowing of sinusoids. The most common changes in muscle tissues at all concentrations of CYP were nuclear proliferation and congestion. Besides, 10 ppm propolis on gill, liver, muscle tissues of rainbow trouts showed significant therapeutic effects. Histological analysis revealed that propolis may serve as an antitoxic agent against pesticide toxicity.

Keywords: Cypermethrin, Propolis, Gill, Liver, Muscle, Histopathology, Rainbow trout

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Introduction

Pesticides are one of the largest groups of toxic substances used in agricultural areas to control insect vectors infesting human agroculture products (Begum, 2004). Accumulation of pesticides in organisms in contaminated water is an important aspect of environmental awareness, because it may affect all members of the food chain, including fish (Tarras-Wahlberg *et al*., 2001). In addition, chemical substances in the aquatic environment not only cause certain injury on organisms but also affect various activities of fish and other organisms in aquatic areas, and even may be killed (Palaniappan *et al*., 2008). The use of synthetic pyrethroid insecticides has been increasing steady in recent times. These chemicals have been reported to be potentially toxic for mammals (Muthuviveganandavel *et al*., 2008). Cypermethrin (CYP) is one of the most toxic pyrethroids (Datta and Kaviraj, 2003a). CYP (R,S)a cyano-3-phenoxybenzyl -2,2-dimethyl (1R,1S)-cis, trans-3-(2,2-dichlorovinyl-cyclopropane carboxylate) is a pyrethroid insecticide which is broadly used for pest control in industrial and agricultural areas because of its low environmental persistence and toxicity (Singh and Singh, 2008). It is highly toxic for the aquatic organisms including fish. The LC_{50} (96 h) value of this pesticide in the blood of rainbow trout is 0.0082 ppm. The metabolism and excretion of CYP in fish is slower than in mammals and birds (Atamanalp *et al*., 2002). Free radicals are important source of toxic effects of pesticides and other environmental toxic agents. Pesticides cause oxidative stress, leading to the production of free radical in cells (Gulhan *et al*., 2012). Antioxidants play an important role in counteracting free radical induced damage to macromolecules. Propolis is the most widely cited antioxidant that prevent oxidative damage to cell membrane induced by radicals (Beyraghdar Kashkooli *et al*., 2011; Gulhan *et al*., 2012; Talas *et al*., 2012). The chemical composition of propolis is mainly composed of flavonoids, terpenes, amino acids and caffeic acid, phenyl esters (Geckil *et al*., 2005). Due to a variety of biological features, an exogenous supply of nutrient such as propolis is desirable for protecting fish from the toxicities of many chemicals. Exposure of aquatic organisms to very low levels or sublethal concentration of pesticides in their environment may result in histological changes in some tissues (Rao, 2006a; Velmurugan *et al*., 2007). Histopathologic changes provide a rapid method to detect effects of damaging in various tissuse and organs (Mallatt, 1985). Gill, liver and muscle are suitable organs for histological study in order to determine the effect of tissue damage (Korkmaz *et al*., 2009).

Pyrethroids are extremely lipophilic and likely to be strongly absorbed by the gills, even at very low concentrations (Smith and Stratton, 1986). Tissue damages caused by water-borne pollutants can be easily monitored because fish gills continuously come into contact with the environment. Dyspnea is one of the early indications of pesticide poisoning (Murty, 1986). Histological researches on fish organs, particularly liver, continuously proved to be remarkably sensitive tool to

reveal detrimental effects in fish induced by toxic agents (Mallatt, 1985; Cengiz and Unlu, 2006).

The objective of the current study was to investigate protective and therapeutic effects of propolis against toxicity of CYP at sublethal concentrations on histological changes in gill, liver and muscle tissues of *Oncorhynchus mykiss*.

Materials and metods

Experimental animals

In experiment, rainbow trouts (*Oncorhyncus mykiss*), with an average body weight of 240-250 g and length of 29.25±3.94 cm, were collected from a freshwater source purchased from Camardi, Ecemis fish farm (Nigde, Turkey). Fish were brought to laboratory within 30 min in plastic tanks with sufficient air. 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. Fish are widely experimental models to evaluate the health of aquatic ecosystems and toxicologic status. Physical and chemical properties of the water are depicted in Table 1.

Table 1: Physico-chemical parameters of the water used in the experiment.

Parameter (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	7.7 ± 0.1	7.7 ± 0.1

Preparation of propolis extractive solution

Propolis extraction methods may influence its activity, because different solvents solubilize and extract different compounds. The most common extracts used in biological assays are ethanol, methanol and water. In the present work, propolis was obtained from a farm at Kocaavsar village in Balikesir, Turkey. Propolis was dissolved in ethanol as 30%, protected from light and moderately shaken for 1 day at room temperature. Afterwards, the extracts were filtered twice, dried and stored in sealed bottles at 4°C until use (Mani *et al*., 2006).

Experimental design

10 ppm treatment (propolis was treated to the animals in 10 ppm), 20 ppm treatment (propolis was treated to the animals in 20 ppm), 30 ppm treatment (propolis was treated to the animals in 30 ppm). In this 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 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 not exposed as control group were used. Each experimental group designed include 8 fish with four replicates. A total of 80 fish were used in this study. Each rainbow trout was weighed just before starting the study. Applications were carried out to the animals for 96 h and not fed for 12 h before in the study. Fish were fed to Excel Pond trade mark pellet feed during experiments. After all of application for 96 hours, fish were anaesthetised with clove oil (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.

Preparation of tissue samples for histological analysis

After expposure period, all of the experimental and control fishes were killed for histopathological examination at the end of 96 h. Gill, liver and muscle tissues were fixed in 10% neutral formalin for 24 h. Fixed tissues were washed in running tapwater for 24 and dehydrated properly through ascending series of ethanol. Then the tissues were cleared with xylene and embedded in paraffin wax. In order to specify the thickness of serial section for histological purpose, generally sections were cut at 4-5 μm thickness and stained with hematoxylin and eosin for light microscopic examination, and sections were examined for investigation of histopathological lesions. Histological preparations were randomly examined three times, and the results from each observation were then combined for the final data.

Results

Gill tissues of control, propolis and CYP+propolis groups

No histopathological changes were observed in the gill tissues of fish in control group. The structural details of gill tissues of rainbow trouts in control group are shown in fig.1.a. The most common changes at the three concentrations (10, 20, 30 ppm) of propolis were shorting of secondary lamellae, fusion and vacuolization. The histological changes noticed in the propolis groups are shown in fig.1.b, d. Fusion of secondary lamellae was noticed in fish treated with 10 ppm propolis for 96 h (Fig.1.b).

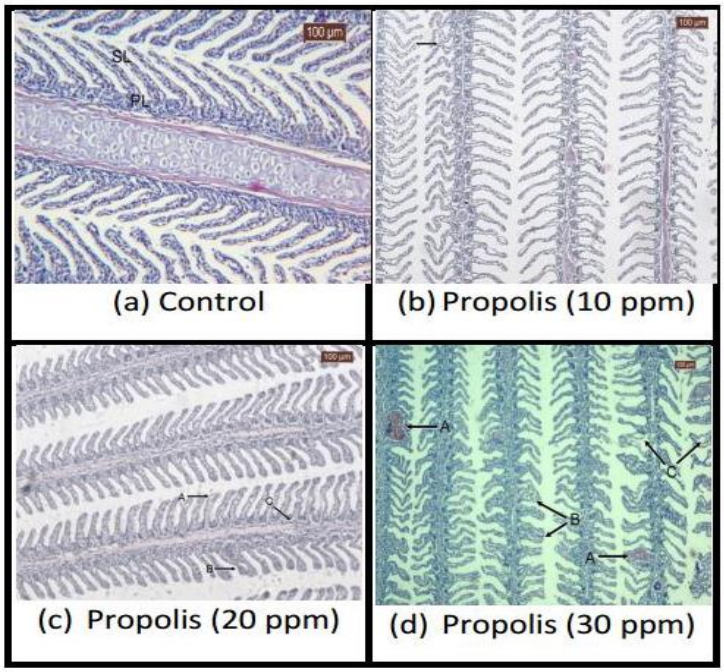

Figure 1: (a). Gill structure of control fish. PL: Primary lamellae, SL: Secondary lamellae H&E, 100×.
(b). 10ppm propolis for 96 h. Fusion of the secondary lamellae. (↑). H&E, 100×.
(c). 20ppm propolis for 96 h. (A) Vacuolization (B) Fusion of the secondary lamellae, (C) Thinning of the secondary lamellae, Cartilage damage, H&E, 100×.
(d). 30 ppm propolis for 96 h. (A) Necrosis (Teleangiocctaxine). (B) Combination of secondary lamellae, (C) Vacuolization H&E, 100×.

Vacuolization, fusion of secondary lamellae, thinning of secondary lamellae, cartilage damage were observed in fish of 20 ppm propolis group (Fig.1.c). Necrosis (Teleangioectasiae), combination of secondary lamellae and vacuolization were observed in fish applied to 30 ppm propolis for 96 h (Fig.1.d). Histopathological results indicated that gill was the primary target tissue affected by CYP. The most common changes at the three concentrations (0.0041, 0.0082, 0.0123 ppm) of CYP were reduced thickness and shorting of secondary lamellae. The histological changes noticed in fish exposed to the CYP concentrations are shown in fig.2, b-d. Shorteng and thinning of secondary lamellae were noticed in fish exposured to 0.0041 ppm CYP for 96 h (Fig.2.b). A combination of shorting of secondary lamellae, thickening and vacuolization were observed in animals exposued to 0.0082 ppm CYP for 96 h (Fig.2.c). Necrosis (Teleangioectasiae), and a combination of secondary lamellae and cartilage damage were noticed in rainbow trout exposued to 0.0123 ppm CYP for 96 h (Fig.2.d). In the groups administered CYP and propolis together (10 ppm) (CYP I+Propolis, CYP II+Propolis, CYP III+ propolis); there were important changes in the histopathology of gill compared with only CYP administered groups (CYP I, CYP II, CYP III). Vacuolization was noticed in fish exposed to 0.0041 ppm CYP+10 ppm propolis for 96 h (Fig.3.b). Reduced thickness of secondary lamellae and

cartilage damage were noticed in fish applied to 0.0082 ppm CYP + 10 ppm propolis (Fig.3.c). Cartilage damage and fusion of secondary lamellae in fish of 0.0123 ppm CYP + 10 ppm propolis group were observed (Fig.3.d).

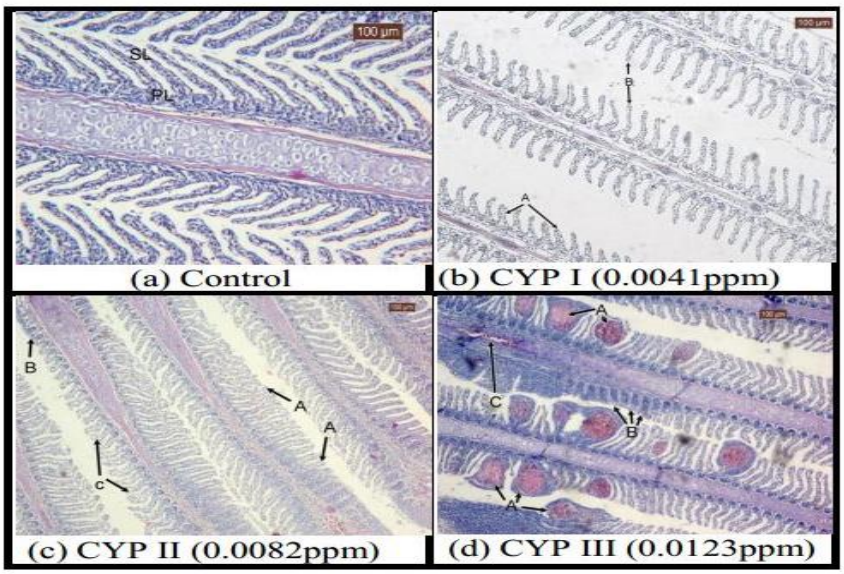

Figure 2: (a). Gill structure of control fish. PL: Primary lamellae, SL: Secondary lamellae H&E, 100×
(b). 0.0041 ppm CYP for 96 h. (A) Shortening of secondary lamellae (B) Thinning of the secondary lamellae, H&E, 100×
(c). 0.0082 ppm CYP for 96 h. (A) Combination of secondary lamellae (B) Shortening of secondary lamellae and thickening (C) Vacuolization, H&E, 100×
(d). 0.0123 ppm CYP for 96 h. (A) Necrosis (Teleangioectasiac) (B) Combination of secondary lamellae (C) Cartilage damage H&E, 100×.

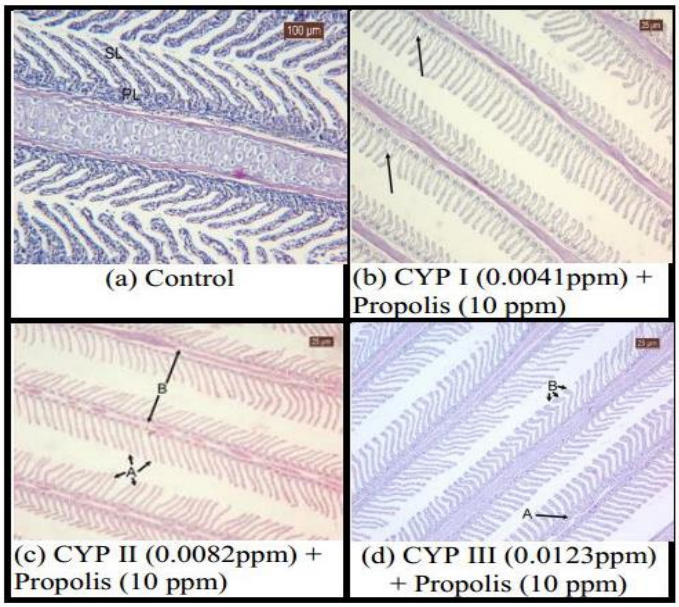

Figure 3: (a). Gill structure of control fish. PL: Primary lamellae, Sl: Secondary H&E, 100×.

(b). 0.0041 ppm CYP+10ppm propolis for 96 h, Vacuolization, (↑), H&E, 40×.

(c). 0.0082 ppm+10ppm propolis for 96 h, (A) Thinning of the secondary lamellae (B) Cartilage damage, H&E, 40×.

(d). 0.0123 ppm CYP+10ppm propolis for 96 h, (A) Cartilage damage (B) Fusion of the secondary lamellae, H&E, 40×.

Liver tissues of control, propolis and CYP+propolis groups

There was no histological change in the liver tissues of control fish. Structural details of liver tissues of fish in control group are shown in fig.4.a. The histological changes noticed in propolis groups are shown in Fig.4.b-d. Infiltration of mononuclear cells was observed in 10 and 30 ppm propolis groups for 96 h (Fig.4.b,d). Congestion, narrowing of sinusoids and hydropic degeneration in the liver tissues of fish treated with30 ppm propolis are shown in fig.1.d. Congestion in the group treated with 20 ppm propolis was observed (Fig.4.c). In liver tissues of fish exposed to three CYP concentrations for 96 h significant changes were seen. Congestion and infiltration of mononuclear cells occured in all the concentrations of CYP for 96 h (Fig.5.b-d). In the groups administered CYP and propolis (10 ppm) together (CYP I+Propolis, CYP II+Propolis, CYP III+Propolis); there were important differences in the histopathology of liver compared to those administered with CYP only (CYP I, CYP II, CYP III). Narrowing of sinusoids and hydropic degeneration were noticed in fish applied with 0.0041 ppm CYP + 10 ppm propolis for 96 h (Fig.6.b). Necrosis, narrowing of sinusoids and hydropic degeneration were observed in fish applied to 0.0082 ppm CYP + 10 ppm propolis for 96 h (Fig.6.c). Congestion and infiltration of mononuclear cells were determined in fish applied with 0.0123 ppm CYP+10 ppm propolis for 96 h (Fig.6.d).

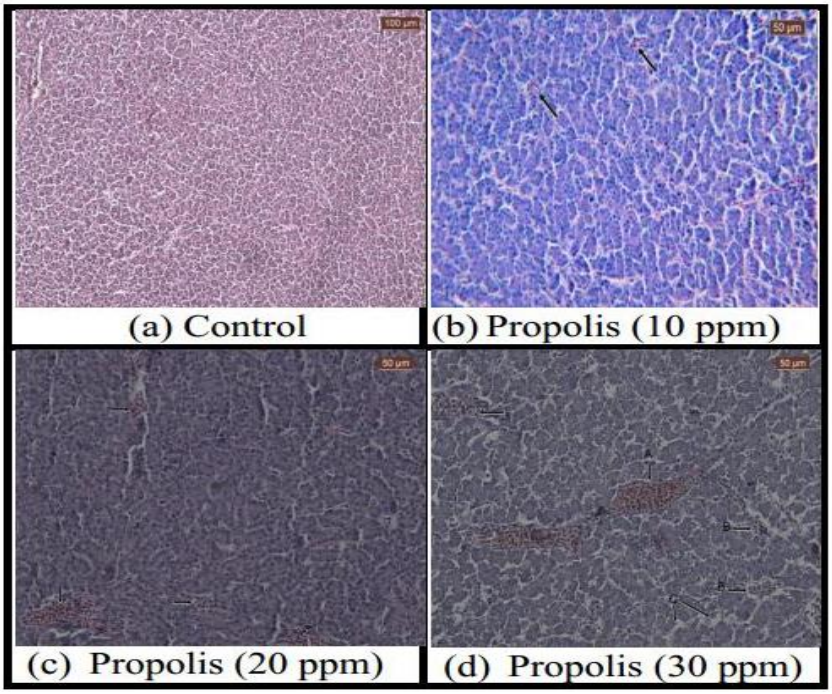

Figure 4: (a). Liver structure of control fish, H&E, 200×.

(b). 10 ppm propolis for 96 h. Infiltration of mononuclear cell, (↑), H&E, 200×.

(c). 20 ppm propolis for 96 h. Congestion, (↑), H&E, 200×.

(d). 30 ppm propolis for 96 h. (A) Congestion (B) Infiltration of mononuclear cell (C) Narrowing of sinusoids and hydrophic degeneration, H&E, 200×.

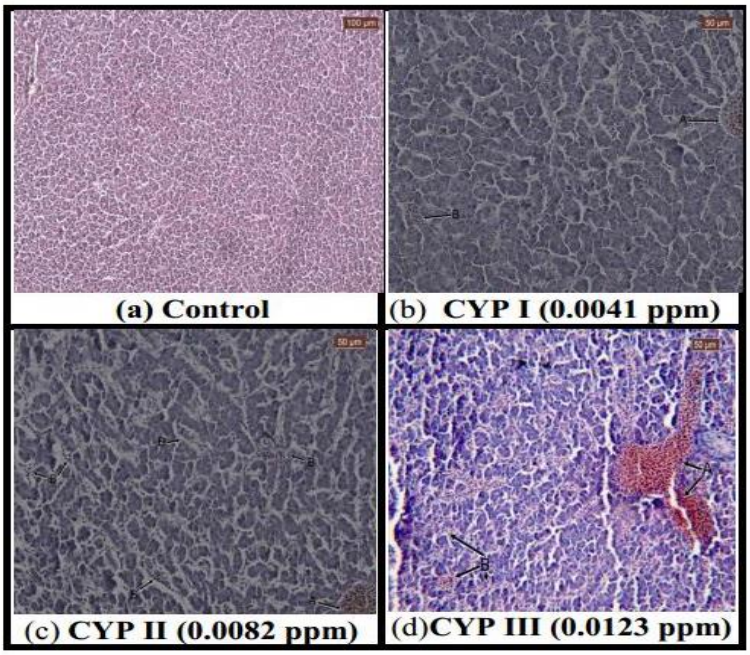

Figure 5: (a). Liver structure of control fish H&E, 200×.

(b). 0.0041 ppm CYP for 96 h. (A) Congestion (B) Infiltration of mononuclear cell H&E, 200×.

(c). 0.0082 ppm CYP for 96 h. (A) Congestion (B) Infiltration of mononuclear cell H&E, 200×.

(d). 0.0123 ppm CYP for 96 h. (A) Congestion (B) Infiltration of mononuclear cell H&E, 200×.

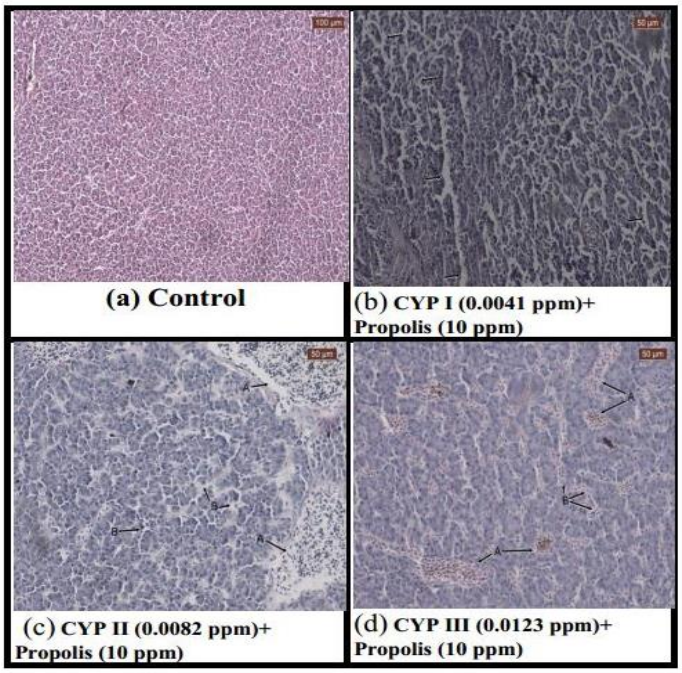

Figure 6: (a). Liver structure of control fish, H&E, 200×.
(b). 0.0041 ppm CYP + 10 ppm propolis for 96 h, Narrowing of sinusoids and hydrophic degeneration, (↑), H&E, 200×.
(c). 0.0082 ppm+10 ppm propolis for 96 h, (A) Necrosis (B) Narrowing of sinusoids and hydrophic degeneration, H&E, 200×.
(d). 0.0123 ppm CYP + 10 ppm propolis for 96 h, (A) Congestion (B) Infiltration of mononuclear cell, H&E, 200×.

Muscle tissues of control, propolis and CYP+propolis groups

There was no histological change in the muscle tissues of fish in control group. Structural details of muscle tissues of fish in control group are shown in Fig.7.a. The histological changes in muscle tissues of propolis groups are shown in fig.7.b-d. Nuclear proliferations in all of the concentrations of propolis (10, 20 and 30 ppm) for 96 h, were noticed (Fig.7.b-d). There were nuclear proliferations in the muscle tissues of fish exposed to 0.0041 and 0.0123 ppm CYP concentrations for 96 h (Fig.8.b,d). Nuclear proliferation and difference in size of muscle fibers were observed in 0.0082 ppm CYP group (Fig.8.c). In the groups administered CYP and propolis (10 ppm) together (CYP I+Propolis, CYP II+Propolis, CYP III+Propolis); there were important changes in the histopathology of muscle tissues compared to CYP only groups (CYP I, CYP II, CYP III). The most common changes at all groups administered of CYP and propolis together (10 ppm) (CYP I+Propolis, CYP II+Propolis, CYP III+propolis) were nuclear proliferation (Fig.9. b-d). Also, congestion in CYP II+propolis (10 ppm) group was determined (Fig.9.c).

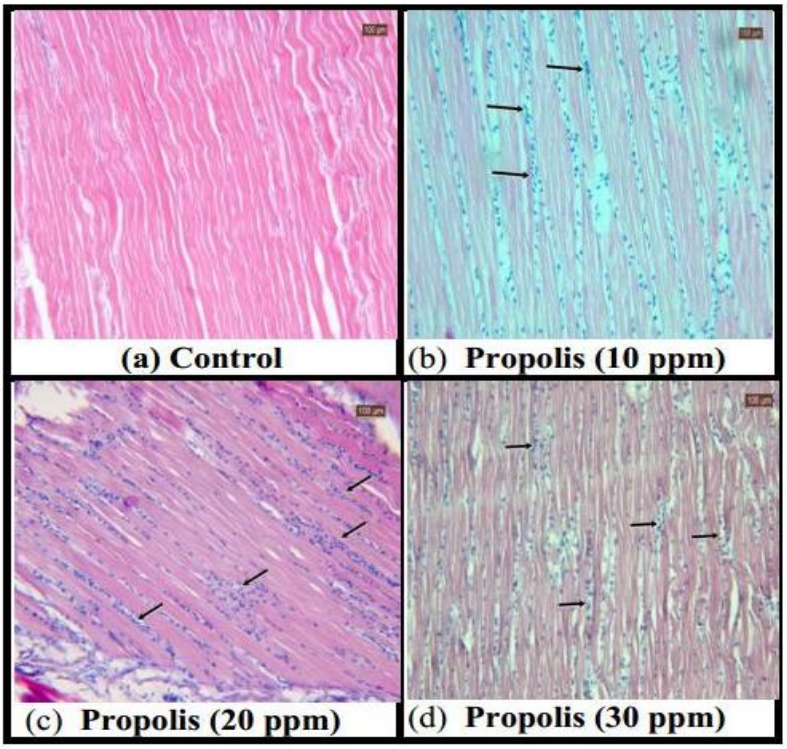

Figure 7: (a). Muscle structure of control fish, (↑), H&E, 100×.
(b). 10 ppm propolis for 96 h. Nuclear proliferation, (↑), H&E, 100×.
(c). 20 ppm propolis for 96 h. Nuclear proliferation, (↑), H&E, 100×.
(d). 30 ppm propolis for 96 h. Nuclear proliferation, (↑), H&E, 100×.

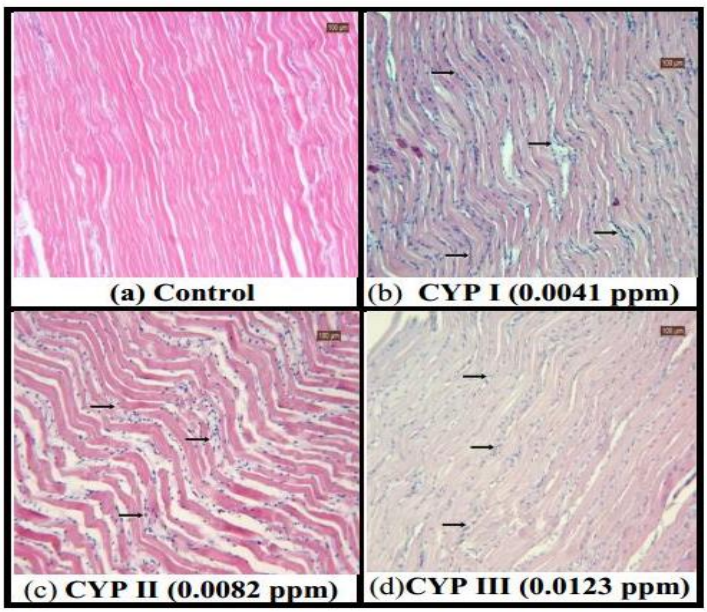

Figure 8: (a). Muscle structure of control fish. (↑), H&E, 100×.
(b). 0.0041 ppm CYP for 96 h. Nuclear proliferation, (↑), H&E, 100×.
(c). 0.0082 ppm CYP for 96 h. Difference in size of muscle fibers, (↑), H&E, 100×.
(d). 0.0123 ppm CYP for 96 h. Nuclear proliferation, (↑), H&E, 100×.

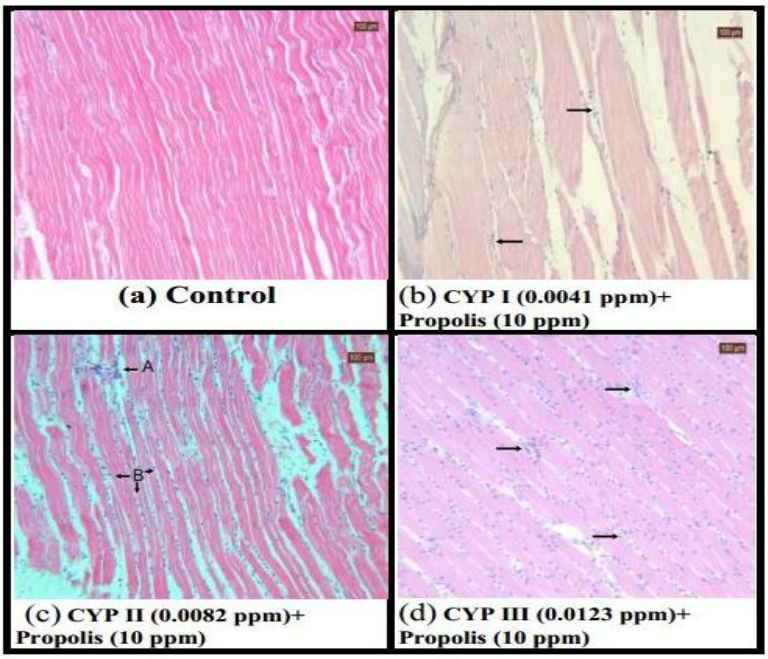

Figure 9: (a). Muscle structure of control fish. (↑), H&E, 100×.
(b). 0.0041 ppm CYP + 10 ppm propolis for 96 h, Nuclear proliferation, (↑), H&E, 100×.
(c). 0.0082 ppm + 10 ppm propolis for 96 h, (A) Congestion (B) Nuclear proliferation, H&E, 100×.
(d). 0.0123 ppm CYP + 10 ppm propolis for 96 h, Nuclear proliferation, (↑), H&E, 100×.

Discussion

Wastewater contains a large-scale of organic pollutants, including organochlorine compounds (cypermethrin, deltamethrin, DDT), organometallic compounds and many metal ions, including cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg) and lead (Pb) (Livingstone *et al*., 1992). All of these toxic agents are powerful oxidants. Since the discovery of the importance of free radical reactions and the mechanisms of toxicity of many harmful compounds (xenobiotics), there have been great interest on investigating pro-oxidant and antioxidant processes, mainly in living organism (Gulhan *et al*., 2012). It is known that pollutants stimulate ROS production and the resulting oxidative damage may be an indicator of toxic damage in aquatic organisms exposed to contamination (Livingston, 2001). ROS produced in living organisms are detoxified via antioxidant defense systems. The antioxidant systems, including nonenzymatic antioxidants such as vitamins, phenolic compounds, flavonoids in aquatic organisms have been investigated (Talas *et al*., 2012; Gulhan *et al*., 2012). Gills generally absorb water-soluble pesticides and liposoluble foreign compounds. Thus, the organophosphate compounds can easily pass through cell membrane systems of rainbow trout. Consequently, biotransformation of insecticides occur in gills, causing poisoning of cells, and damaging tissues (Fanta *et al*., 2003). Respiratory distress is

one of the early symptoms of pesticide intoxication (McDonald, 1983). Extreme absorbtion of cypermethrin through gills makes the fish as an undefended target for toxic damage (Srivastav *et al*., 1997). Histopathologic changes provide an important method to detect effects of damaging in tissues and organs (Mallatt, 1985).

In this study, the most common histological changes at all concentrations of propolis were shorting secondary lamellae, fusion and vacuolization. Similar findings such as the lifting of epithelial layer from gill lamellae, necrosis, shorting and degeneration of secondary lamellae, and clupshaped lamellae in the gills of *Lepistes reticulatus* exposed to cyphenothrin were observed by Erkmen *et al*. (2000). Aneurism, epithelial necrosis, secondary lamellae showing fusion, lifting of epithelium have also been observed in *Gambusia affinis* (Cengiz and Unlu, 2003). Jiraungkoorskul et al. reported the fusion and thickening of secondary lamella in Nile tilapia after three months following exposure to 5 and 15 mg/l glyphosate herbicide (Jiraungkoorskul *et al*., 2003). Yildirim *et al*. reported the hyperemia, fusion of the secondary lamella and telangiectasis after exposure to 5 µg/l deltamethrin for 48 h (Yildirim *et al*., 2006). Fanta *et al*. (2003) studied the histopathology of *Corydoras paleatus* contaminated with sublethal levels of organophosphorus pesticides in water and food. They examined effects of contamination with a sublethal concentration of methyl parathion, through water or food in tissues. These researchers showed the epithelial hyperplasia and oedema on gill tissues (Fanta *et al*., 2003). Observed necrosis, shorting of secondary lamellae and thickening, vacuolization of gill epithelium are direct responses to the toxic effects of CYP. The distance to reach the blood stream of toxicant is increased . Thus, the shorting, reduced thickness and a combination of secondary lamellae and vacuolization are increased in fish gills. Gill necrosis might serve as a defensive mechanism leading to a decrease in the respiratory surface and an increase in the toxicant blood diffusion distance (Majeed *et al*., 2014).

In the present study, there were important differences in the histopatology of gill in groups co-administered with CYP and propolis compared to groups administered various concentrations of CYP. Vacuolization was noticed in gill of fish applied with 0.0041 ppm CYP+10 ppm propolis for 96 h. Cartilage damage were noticed in group administrated with 0.0082 ppm CYP+10 ppm propolis for 96 h. We noticed a decrease in damage of gill tissues after the administration of propolis. Reduction of the damage is thought to be as a result of therapeutic effects of propolis and its components. Datta and Kaviraj (2003b) estimated efficiency of supplementation of ascorbic acid to eleminate the stress of deltamethrin on freshwater catfish *Clarias gariepinus*. *C. gariepinus* displayed various symptoms of stress when exposed to deltamethrin. Korkmaz *et al*. (2009) studied 0.22 and 0.44 µg/l cypermethrin + control diet, 0.22 and 0.44 µg/l cypermethrin + ascorbic acid supplement diet for 20 days in Nile

tilapia. These results exhibited that ascorbic acid may serve as an antitoxic agent against pesticide toxicity in fish.

Liver is the organ that contains the major concentration of pesticide residues and help in determining the levels of damage as a consequence of this process (Fanta *et al*., 2003).

In present study, infiltration of mononuclear cells in liver tissues of fish treated with 10 and 30 ppm propolis was noticed. In addition, congestion, narrowing of sinusoids and hydropic degeneration in 30 ppm propolis group was observed. Only congestion in 20 ppm propolis group was observed. The congestion and infiltration of mononuclear cells in all concentrations of cypermethrin for 96 h, were observed.

Similar observations have been carried out by several researchers. Cengiz *et al*. (2001) emphased that there were hepatic lesions, including degeneration, hypertrophy, sinusoids enlargement, hemorrhage, pycnosis position of nuclei, vacuolization of cell cytoplasm, infiltration of mononuclear lymphocyte in fish exposed to pollutants. In another study, cloudy swelling, focal necrosis and vacuolization have been reported in the *C. paleatus* exposed to methyl parathion (Fanta *et al*., 2003). Cengiz and Unlu (2006) determined the hypertrophy of hepatocytes, increasing kupffer cells, circulatory disturbance, narrowing of sinusoids and focal necrosis in the liver of *G. affinis* exposed to deltamethrin. Korkmaz *et al*. (2009) showed the histopathological lesions such as, nuclear pycnosis, narrowing of sinusoids, congestion, hypertropy of hepatocytes, vacuolar degeneration, necrosis and fatty degeneration in the tissue of nile tilapia induced to cypermethrin in their study . In other study, hyperplasia, disintegration of hepatic mass, focal coagulative necrosis were seen in *Labeo rohita* exposed to cypermethrin (Sarkar *et al*., 2005).

In the application of cypermethrin and propolis (10 ppm), there were important differences in the histopathology of liver compared with only administered to various concentrations of CYP. Narrowing of sinusoids and hydropic degeneration were noticed in 0.0041 ppm CYP + 10 ppm propolis group. Necrosis, narrowing of sinusoids and hydropic degeneration were observed in fish applied with 0.0082 ppm CYP + 10 ppm propolis for 96 h. Congestion and infiltration of mononuclear cells were determined in 0.0123 ppm CYP + 10 ppm propolis group. The treatment by propolis having antioxidant properties were observed in damaged tissues of fish by different concentrations of CYP. The histological findings of other researchers showed parallelism with the results of our study (Datta and Kaviraj, 2003b; Korkmaz *et al*., 2009).

Fish fillet has an important role in the human diet. The muscle of rainbow trout is significant in determining the freshness of fillet quality in values above or below optimal condition. Stress and excessive muscle activity lead to insufficient amount of oxygen. Fish fillet may be affected by

toxic agents such as CYP and be damaged structurally.

In this study, the various concentrations of propolis (10, 20 and 30 ppm) caused the nuclear proliferations. In the muscle tissues of fish exposed to 0.0041 and 0.0123 ppm of CYP concentrations, only nuclear proliferations were observed. Nuclear proliferation and different size of muscle fibers were seen in 0.0082 ppm CYP group.

Our findings in muscle tissues exposed to cypermethrin are also in accordance with results of other studies (Rabitto *et al*., 2005; Korkmaz *et al*., 2009). In the application of CYP and propolis; the most common change was nuclear proliferations. In addition, congestion was determined in CYP II + propolis group.

Biomolecules (proteins, nucleic acids, lipids, and glycogen) are among the structures affected by toxic agents, which may cause histological changes. Proteins contribute to the structure and physiology of the cells and play an important role in cell metabolism. The decrease in protein content is presumably a physiological adaptation of fish to oxidant stress caused by the pesticide. In order to overcome the stress factors, fish requires high energy. This energy demand might lead to the stimulation of protein catabolism (Sancho *et al*., 1997). This fact can be correlated with increasing oxidative stress in *Oncorhyncus mykiss* exposed to cypermethrin. Histologic changes as a result of decreasing protein in *Clarias batrachus* exposed to fenvalerate were declareted by Tripathi *et al*. (2002) and other researchers (Das and Mukherjee, 2003; David *et al*., 2004). The decreasing protein levels in tissues may be a role of compensatory mechanism under cypermethrin stress.

Depletion of glycogen in the tissues is an indication of typical stress response of the fish challenging with pesticides (Rao, 2006b). Decrease in muscle glycogen content of *Channa punctatus* exposed to lambda cyhalothrin and permethrin were reported by Saxena and Gupta (2005). The reductions in liver and gill glycogen content were also observed in *C. batrachus* exposed to cypermethrin (Begum, 2005). In our study, histological changes occurred by exposure to cypermethrin may consider as a result of damaging effects on the biomolecules such as proteins, lipids of the fish.

In conclusion, the propolis and its polyphenolic compounds are effective to reduce histological changes originated from oxidative stress. Propolis has been used from early times, a better understanding of the actions of its polyphenolic compounds on the immune response will provide a scientific basis for the better therapeutic application and reorganize its use. This work may shed light on investigations about biological activities of new extracts of natural products such as propolis on aquatic organisms.

Acknowledgements

Nigde University Research Fund (FEB 2007/13) is gratefully acknowledged for support of this work.

References

Atamanalp, M., Yanik, T., Haliloglu, H. I. and Aras, M.S., 2002. Alterations in the haematological parameters of rainbow trout, *Oncorhynchus mykiss*, exposed to cypermethrin. *Israel Journal of Earth Sciences,* 54(**3**), 99-103.

Begum, G., 2004. Carbofuran insecticide induced biochemical alterations in liver and muscle tissues of the fish *Clarias batrachus* (linn) and recovery response. *Aquatic Toxicology,* 66, 83-92.

Begum, G., 2005. In vivo biochemical changes in liver and gill of *Clarias batrachus* during cypermethrin exposure and following cessation of exposure. *Pesticide Biochemistry and Physiology,* 82(**3**), 185-196.

Beyraghdar Kashkooli, O., Dorcheh, E. E., Mahboobi-Soofiani, N. and Samie, A., 2011. Long-term effects of propolis on serum biochemicalparameters of rainbow trout (*Oncorhynchus mykiss*). *Ecotoxicology and Environmental Safety,* 74, 315-318.

Cengiz, E.I., Unlu, E. and Balci, K., 2001. The histopathological effects of Thiodan® on the liver and gut of mosquitofish, *Gambusia affinis. Journal of Environmental Science and Health B,* 36(**1**), 75–85.

Cengiz, E.I. and Unlu, E., 2003. Histopathology of gills in mosquitofish. *Gambusia affinis* after long-term exposure to sublethal concentrations of malathion. *Journal of Environmental Science and Health B,* 38(**5**), 581-589.

Cengiz, E.I. and Unlu, E., 2006. Sublethal effects of commercial deltamethrin on the structure of the gill, liver and gut tissues of mosquitofish, *Gambusia affinis*: A microscopic study. *Environmental Toxicology and Pharmacology,* 21, 246-253.

Das, B.K. and Mukherjee, S.C., 2003. Toxicity of cypermethrin in *Labeo rohita* fingerlings: biochemical enzymatic and haematological consequences. *Comparative Biochemistry and Physiology C,* 134, 109-121.

Datta, M. and Kaviraj, A., 2003a. Acute toxicity of the synthetic pyrethroid deltamethrin to freshwater catfish *Clarias gariepinus*. *Bulletin of Environmental Contamination and Toxicology,* 70(**2**), 296-299.

Datta, M. and Kaviraj, A., 2003b. Ascorbic acid supplementation of diet for reduction of deltamethrin induced stress in freshwater catfish *Clarias gariepinus*. *Chemosphere,* 53(**8**), 883-888.

David, M., Mushigeri, S. B., Shivakumar, R. and Philip, G.H., 2004. Response of *Cyprinus carpio* (Linn) to sublethal concentration of cypermethrin: alterations in protein

metabolic profiles. *Chemosphere,* 56 (**4**), 347-352.

Erkmen, B., Caliskan, M. and Yerli, S. V., 2000. Histopathological effects of cyphenothrin on the gills of *Lebistes reticulatus*. *Veterinary and Human Toxicology,* 42(**1**), 5-7.

Fanta, E., Rios, F.S.A., Romao, S., Vianna, A.C.C. and Freiberger, S., 2003. Histopathology of the fish *Corydoras paleatus* contaminated with sublethal levels of organophosphorus in water and food. *Ecotoxicology and Environmental Safety,* 54, 119-130.

Geckil, H., Ates, B., Durmaz, G., Erdogan, S. and Yilmaz, I., 2005. Antioxidant, free radical scavenging and metal chelathing characeristics of propolis. *American Journal of Biochemistry and Biotecnology,* 1(**1**), 27-31.

Gulhan, M.F., Duran, A., Selamoglu Talas, Z., Kakoolaki, S. and Mansouri, S.M., 2012. Effects of propolis on microbiologic and biochemical parameters of Rainbow trout (*Oncorhynchus mykiss*) after exposure to the pesticide. *Iranian Journal of Fisheries Science,* 11(**3**), 490-503.

Jiraungkoorskul, W., Upatham, E.S., Kruatrachue, M., Sahaphong, S., Vichasri- Grams, S. and Pokethiyiyook, P., 2003. Biochemical and histopathological effects of glyphosate herbicide on Nile tilapia (*Oreochromis niloticus*). *Environmental Toxicology,* 18, 260-267.

Korkmaz, N., Cengiz, E.I., Unlu, E., Uysal E. and Yanar, M., 2009. Cypermethrin-induced histopathological and biochemical changes in Nile tilapia (*Oreochromis niloticus*), and the protective and recuperative effect of ascorbic acid. *Environmantal Toxicology and Pharmacology,* 28, 198–205.

Livingstone, D. R., Lips, F., Garcia Martinez, P. and Pipe, R. K., 1992. Antioxidant enzymes in digestive gland of the common mussel, *Mytilus edulis* L. *Marine Biology,* 112, 265-276.

Majeed, S.A., Nambi, K.S.N., Taju, G., Sarath Babu, V., Farook, M.A. and Sahul Hameed, A.S., 2014. Development and characterization of a new gill cell line from air breathing fish *Channa striatus* (Bloch 1793) and its application in toxicology: Direct comparison to the acute fish toxicity. *Chemosphere,* 96, 89-98.

Mallatt, J., 1985. Fish gill structural changes induced by toxicants and other irritants: a statistical review. *Canadian Journal of Fisheries and Aquatic Sciences,* 42(**4**), 630-648.

Mani, F., Damasceno, H. C. R., Novelli, E. L. B., Martins, E. A. M. and Sforcin, J.M., 2006. Propolis: Effect of different concentrations, extracts and

intake period on seric biochemical variables. *Journal of Ethnopharmacology*, 105, 95-98.

McDonald, D.G., 1983. The effects of H^+ upon the gill of fresh water fish. *Canadian Journal of Zoology*, 61, 691-703.

Murty, A.S., 1986. Toxicity of pesticides to fish, vol. 2. CRC Press, Boca Raton, Florida, 160P.

Muthuviveganandavel, V., Muthuraman, P., Muthu, S. and Srikumar, K., 2008. A study on low dose cypermethrin induced histopathology, lipid peroxidation and marker enzyme changes in male rat. *Pesticide Biochemistry and Physiology*, 91, 12-16.

Mylonas, C.C., Cardinaletti, G., Sigelaki, I. and Polzonetti-Magni, A., 2005. Comparative efficacy of clove oil and 2-phenoxyethanol as anesthetics in the aquaculture of European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) at different temperatures. *Aquaculture*, 246(**1-4**), 467-481.

Palaniappan, P.L.R.M., Sabhanayakam, S., Krishnakumar, N. and Vadivelu, M., 2008. Morphological changes due to Lead exposure and the influence of DMSA on the gill tissues of the freshwater fish, *Catla catla*. *Food and Chemical Toxicology*, 46, 2440-2444.

Rabitto, I.S., Alves Costa, J.R.M., Silva de Assis, H.C., Pelletier, E., Akaishi, F.M., Anjos, A., Randi, M.A.F. and Oliveira, R.C.A., 2005. Effects of dietary Pb(II) and tributyltin on neotropical fish, *Hoplias malabaricus*: histopathological and biochemical findings. *Ecotoxicology and Environmental Safety*, 60, 147-156.

Rao, J.V., 2006a. Biochemical alterations in euryhaline fish, *Oreochromis mossambicus* exposed to sublethal concentrations of an organophosphorus insecticide, monocrotophos. *Chemosphere*, 65(**10**), 1814-1820.

Rao, J.V., 2006b. Toxic effects of novel organophosphorus insecticide (RPR-V) on certain biochemical parameters of euryhaline fish, *Oreochromis mossambicus*. *Pesticide Biochemistry and Physiology*, 86(**2**), 78-84.

Sancho, E., Ferrando, M.D. and Andreu, E., 1997. Sublethal effects of an organophosphate insecticide on the European eel, *Anguilla anguilla*. *Ecotoxicology and Environmental Safety*, 36, 57-65.

Sarkar, B., Chatterjee, A., Adhikari, S. and Ayyappan, S., 2005. Carbofuran and cypermethrin-induced histopathological alterations in the liver of *Labeo rohita* (Hamilton) and its recovery. *Journal of Applied Ichthyology*, 21(**2**), 131-135.

Saxena, K.K. and Gupta, P., 2005. Pyrethroids induced toxicity to muscle glycogen contents in *Channa punctatus*. *Proceeding of the* Indian *National Science Academy*, 75(**2**), 103-106.

Singh, P.B. and Singh, V., 2008. Cypermethrin induced histological changes in gonadotrophic cells, liver, gonads, plasma levels of estradiol-17b and 11-ketotestosterone, and sperm motility in *Heteropneustes fossilis* (Bloch). *Chemosphere*, 72, 422–431.

Smith, T.M. and Stratton, G.W., 1986. Effect of synthetic pyrethroid insecticides on non-target organisms. *Residue Review*, 97, 93-120.

Srivastav, A.K., Srivastav, S.K. and Srivastav, S.K., 1997. Impact of deltamethrin on serum calcium and inorganic phosphate of freshwater catfish. Heteropneustes fossilis. *Bulletin of Environmental Contamination Toxicology*, 59(**5**), 841–846.

Talas, Z.S., Dundar, S.P., Gulhan, M. F., Orun, I. and Kakoolaki, S., 2012. Effects of propolis on some blood parameters and enzymes in carp exposed to arsenic. *Iranian Journal of Fisheries Sciences*, 11(**2**), 405- 414.

Tarras-Wahlberg, N.H., Flachierm, A., Lane, S.N. and Sangfors, O., 2001. Environmental impacts and metal exposure of aquatic ecosystems in rivers contaminated by small scale gold mining: The Puyango River Basin, Sourthen Ecuador. *Science of Total Environment*, 278(**1-3**), 239-261.

Tripathi, G., Harsh, S. and Verma, P., 2002. Fenvalerate-induced macromolecular changes in the catfish, *Clarias batrachus*. *Journal of Environmental Biology*, 23(**2**), 143-146.

Velmurugan, B., Selvanayagam, M., Cengiz, E.I. and Unlu, E., 2007. Histopathology of lambda-cyhalothrin on tissues (gill, kidney, liver and intestine) of *Cirrhinus mrigala*. *Environmental Toxicology and Pharmacology*, 24(**3**), 286–291.

Yildirim, M.Z., Benli, A.C.K., Selvi, M., Ozkul, A., Erkoc, F. and Kocak, O., 2006. Acute toxicity, behavioral changes, and histopathological effects of deltamethrin on tissues (gills, liver, brain, spleen, kidney, muscle, skin) of Nile tilapia (*Oreochromis niloticus* L.) fingerlings. *Environmental Toxicology*, 21, 614-620.