Basic and Clinical
NEUROSCIENCE
May 2013, Volume 4, Number 2

Role of Propolis on Oxidative Stress in Fish Brain

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Article info:
Received: 01 February 2013
First Revision: 20 February 2013
Accepted: 06 March 2013

Key Words:
Brain,
Cypermethrin,
Oxidative Stress,
Propolis,
Rainbow Trout.

ABSTRACT

Introduction: Cypermethrin causes its neurotoxic effect through voltage-dependent sodium channels and integral protein ATPases in the neuronal membrane. Brain and nerve damage are often associated with low residual level of pesticides. In vitro and in vivo studies have also shown that pesticides cause free radical-mediated tissue damage in brain. Propolis has antioxidant properties. The main chemical classes found in propolis are flavonoids and phenolics. Bioflavonoids are antioxidant molecules that play important roles in scavenging free radicals, which are produced in neurodegenerative diseases and aging.

Methods: To determine the protective role of propolis, rainbow trouts were treated with cypermethrin, followed by biochemical analyses of brain tissue. Fish were divided into four groups: control, propolis-treated, cypermethrin-treated, and cypermethrin+propolis-treated.

Results: In fish brains, catalase (CAT) activity decreased ($P \leq 0.001$) and malondialdehyde (MDA) level increased ($P \leq 0.001$) in cypermethrin-treated group compared to control group. In cypermethrin + propolis-treated group CAT activity increased ($P \leq 0.001$) and MDA level decreased ($P \leq 0.001$) compared to cypermethrin group.

Discussion: The results demonstrated that the negative effects, observed as a result of cypermethrin treatment, could be reversed by adding supplementary propolis. Propolis may improve some biochemical markers associated with oxidative stress in fish brain, after exposure to cypermethrin.

1. Introduction

Cypermethrin, a synthetic pyrethroid, is widely used as a pesticide. Like other insecticides, the widespread use of cypermethrin has been associated with adverse effects on nontarget species. Consistent with its lipophilic nature, cypermethrin has been found to accumulate in body fat, skin, liver, kidneys, adrenal glands, ovaries, and brain. Cypermethrin exerts its neurotoxic effect through voltage-dependent sodium channel and integral protein ATPase in the neuronal membrane. In vitro and in vivo studies have also shown that it causes free radical-mediated tissue damage in brain, liver, and erythrocytes (Tao et al., 2008). After use, cypermethrin is released directly into the environment, enters water sources and effects the aquatic ecosystem. Large-scale population declines of many species of birds, mainly fish-eating and bird-eating species, have been attributed to exposure to insecticides through higher order food chain and upward biomagnification of residues (Muthuviveganandavel et al., 2008).

To reduce damage to environment by pesticides, biological systems have developed protective means which include antioxidant molecules. When toxic agents ex-

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haust the endogenous levels of antioxidants, exogenous antioxidative and protective compounds may be substituted to minimize adverse health outcomes (Devillers et al., 2001). The investigation of new antioxidants as potential therapeutic agents is an active field of biochemistry. A variety of organic forms of antioxidant molecules have been studied as natural therapeutic and preventive agents. Propolis, is a phenolic compound which may be capable of preventing apoptosis by decreasing oxidative stress (Kanbur et al., 2009).

Fish is an important aquatic organism. Fish products are an important source of protein for human consumption (Duran and Talas, 2009). Aquatic organisms can provide model systems for investigation of how reactive oxygen species (ROS) damage cellular compounds, how cells respond, how repair mechanisms reverse this damage, and how oxidative stress can lead to disease. Oxidative stress has become an important item for aquatic toxicology.

The most widely used assay for lipid peroxidation is malondialdehyde (MDA) formation which represents the secondary lipid peroxidation product with the thiobarbituric acid reactive substances test. MDA is the final product of lipid peroxidation. The concentration of MDA is a measure of free radical damage to lipids. Lipid peroxides could change the properties of biological membranes, resulting in eventual cell damage (Alirezaei et al., 2012).

In order to minimize such damage, cells have evolved defense systems comprising both enzymatic and nonenzymatic processes. An example of an enzymatic defense system, is the antioxidative enzyme catalase (CAT). Flavonoids comprise a nonenzymatic antioxidant molecule. Flavonoids are potent antioxidants, free radical scavengers and metal chelators. The different antioxidant properties of these compounds help to prevent the irreversible lipid peroxidation which occurs with oxidative stress (Haq et al., 2012).

In this study, rainbow trout (Oncorhynchus mykiss), one of the most popular cultural fish in Turkey, was chosen as the model organism in the human diet. We investigated the effects of the antioxidant propolis on biochemical parameters (MDA and CAT) in brain tissues of farmed rainbow trout.

2. Methods

2.1. Experimental Section

The rainbow trout (Oncorhynchus mykiss) were purchased from Camardı, Ecemis fish farm (Nigde, Turkey). Fish were fed for 15 days in a 8 x 5 x 1.5 m stock aquarium to be acclimatized. After adaptation period, 8 fish were transferred to 200 L water tank filled with natural spring water. Fish used in this study had an average weight of 245.51±5.22 g and length of 29.75 ± 3.81 cm physical and chemical properties of water are depicted in table 1.

Table 1. Some 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 (CaCO3)	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

2.2. Preparation of Propolis Extractive Solution

Method constructing propolis extraction may influence its activity, because different solvents solubilize and extract different compounds. There are three methods commonly used for extraction with ethanol, methanol and water. The chemical composition or propolis is very complex: content of more than 300 components have been identified, and its composition is directly dependent with the composition of the vegetation of the region. Moreover, propolis composition is completely variable creating a problem for the medical use and standardization. In the present work, propolis was collected from a farm at village Kocaavsar in Balikesir, Turkey. Propolis was dissolved to 30 % in ethanol, protected from light and moderately shaken for 1 day at room temperature. Afterward, the extracts were filtered twice, dried and stored in sealed bottles at 4°C until use (Mani et al., 2006).

Biochemical parameters of rainbow trout treated propolis at various doses were investigated, and the effects of 10 ppm propolis were outlined (Talas and Gulhan, 2009).

2.3. Experimental Design

Thirty two rainbow trouts were divided into four groups, each consisting of eight animals. Each rainbow trout was weighted just before the start of the study. Rainbow trout were grown in fresh water supplemented with either, 0.0082 ppm cypermethrin (Atamanalp et al., 2002a; Atamanalp et al., 2002b), 10 ppm propolis (Talas and Gulhan, 2009), or 0.0082 ppm cypermethrin + 10 ppm propolis (Gulhan et al., 2012). Control group was grown in fresh water without any supplementation. The animals in the experimental groups were treated with 0.0082 ppm cypermethrin and/or 10 ppm propolis for 96 h. Fish were fed Excel Pond trade mark pellet feed during experiments. Fish experiments were performed in accordance with the guidelines for approved by the Committee of Animal Experiments at Cumhuriyet University, Sivas, Turkey.

2.4. Preparation of Tissues for Biochemical Analyses

After exposures, 2 mL of blood was obtained from caudal vein of rainbow trout. Brain tissue of fish were removed and frozen in liquid nitrogen. Tissues were stored at −80°C until used. The tissues were separated into two parts for determination of CAT activity or lipid peroxidation. Tissues were weighed and then homogenized in 100 mL of 2 mM phosphate buffer, pH 7.4 using PCV Kinematica Status Homogenizer. Homogenized samples were then sonicated for 1.5 min (30 s sonications interrupted with 30 s pause on ice). Samples were then centrifuged at 12,000g for 15 min at 4°C and supernatants, if not used for enzyme assays immediately, were kept in the deep freeze at −80°C. Supernatants were used for determination of total protein and measurement of CAT activity. The second part of tissues homogenate was used for lipid peroxidation analysis. Tissues were washed three times with ice-cold 0.9% NaCl solution and homogenized in 1.15% KCl. The homogenates were assayed for MDA, the product of lipid peroxidation.

2.5. Protein Assay

Supernatants of tissues were used for determination of total protein. Total protein was quantified by the colorimetric method of Lowry et al. using BSA as the standard (Lowry et al., 1951).

2.6. Analysis of CAT Activity

The CAT activity in the tissues was determined by measuring the decompositon of hydrogen peroxide at 240 nm, according to the method of Aebi. It was expressed as kU/g protein, where k is the first-order rate constant (Aebi, 1984).

2.7. Measurement of MDA Levels

Tissue MDA levels were determined spectrophotometrically using thiobarbituric acid (TBA) solution (Yagi, 1984). The reaction mixture containing tissue homogenate, phosphoric acid, TBA and sulfuric acid was heated 60 minutes in a boiling water bath. After cooling, n-butanol was added and mixed vigorously. The butanol phase was separated by centrifugation and absorbance was measured at 532 nm. For quantification an external standart curve was prepared using 1, 1, 3, 3 tetraethoxypropane. Values were expressed as nmol/g tissue.

2.8. Statistical Analysis

Biochemical data were analyzed with 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 ($P \leq 0.001$).

3. Results

Effects of cypermethrin and propolis on CAT activities and MDA levels on rainbow trout brain is shown

in table 2. CAT activity decreased in the brain tissue of cypermethrin treated fish compare with control group (P≤0.001) (Table 2). There was no statistically significant change (P≥0.001) in CAT activity of propolis treated group compared with control group in the brain tissue of fish (Table 2). There was statistically significant increase in CAT activity of L- cypermethrin+propolis group compared with cypermethrin exposed group (P≤0.001) in the brain tissue of rainbow trout (Table 2).

There was a statistically significant increase (P≤0.001) in MDA after cypermethrin exposure compared with control group. In propolis treated group there was a significant reduction (P≤0.001) of MDA levels compared with control and cypermethrin groups (Table 2). Propolis plus cypermethrin exposure caused a statistically significant decrease (P≤0.001) in MDA levels compared to cypermethrin treated and control groups in the rainbow trout brain tissues (Table 2).

Table 2. Changes on the MDA levels and CAT activities in rainbow trout brain tissues

Groups	MDA (nmol/g tissue ± SEM)	CAT (kU/g protein ± SEM)
Control	17,66±0,72c	0,0125±0,0026a
Cypermethrin	22,86±1,48b	0,0056±0,0003b
Propolis	10,38±0,54a	0,0110±0,0021a
Cypermethrin+propolis	11,78±0,38b	0,0129±0,0015a

All data points are the average of n=8 with ±STDEV.

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abcstatistically significant (P≤0.001)

4. Discussion

Toxicants can have a important role in the development and progression of many disease processes. Exposure to contaminants will depend on the particular dietary and ecological lifestyles of the aquatic organisms. Problems of mutagenicity, teratogenicity, or carcinogenicity with pyrethroids are rare, as they do not appear to pose serious residue problems in food and food products. Membrane phospholipids of aerobic organisms are permanently subjected to oxidant challenges from endogenous and exogenous sources. Peroxidized membranes and lipid peroxidation products represent constant threats to aerobic cells. The most widely used assay for lipid peroxidation is MDA formation as a secondary lipid peroxidation product, with the thiobarbituric acid reactive substances test. The concentration of MDA is the direct evidence of lipid damage caused by free radicals (Orun et al., 2011; Talas and Duran, 2012).

The first line of defense mechanism against damaging effects of ROS are antioxidant enzymes such as CAT which directly scavenges the superoxide radicals and hydrogen peroxide, converting them to less reactive species. Superoxide dismutase catalyzes the dismutation of $O2^{\cdot}-$ to H2O2, and CAT reduces H2O2 to 2 H2O (Atli et al., 2006). Increased level of MDA and a reduction in the activity of CAT were observed in brain tissue of cypermethrin exposed fish. Thisis in agreement with previous reports (Ates et al., 2008; Orun et al., 2008; Ventura-Lima et al., 2009; Orun et al., 2011; Gogebakan et al., 2012; Oliva et al., 2012).

Fish exposed to cypermethrin show a tendency toward decreased antioxidant enzyme activity. In the present study, CAT activity in fish brain was decreased by pesticide exposure. Similar findings have been reported by several researchers (Orun et al., 2008; Ventura-Lima et al., 2009; Orun et al., 2011; Oliva et al., 2012).

Negative correlations were found between CAT activities and MDA levels. Lipid peroxidation, considered a complex self propagating process producing high levels of cell degradation, increases the rigidity (decreases the fluidity) of cellular membranes (Ates et al. 2008; Talas et al. 2008).

Antioxidative effects have been noted with various food and natural products. Most recent studies have shown that natural protective compounds have gained popularity as some of the widely used synthetic pharmaceuticals and therapeutics might have some unexpected adverse effects. Propolis may be a potential natural therapeutic and preventive agent against pesticide damage. propolis. Natural antioxidants are essential for homeostasis in many biological systems. Due to antioxidant and preservative properties of propolis, it may both prolong the physiological functions of some aquatic organisms and contribute to the health benefit of consumers that consume aquatic animals. Increased CAT activity

after propolis treatment in fish exposed to cypermethrin may be related to antioxidant effect of propolis. Our results are in accordance with previous reported results (Ramanathan et al., 2003; Nandi et al., 2008).

Propolis has flavonoids and phenolic compounds. Flavonoids are potent antioxidants, free radical scavengers, and they inhibit lipid peroxidation and exhibit various physiological activities, including antihypertensive and vasodilating activities. In this study, a protective role for propolis was demonstrated.

Propolis is a therapeutical natural substance. It supports the immune system and has antioxidative properties. There are many studies concerning the therapeutic effects of propolis on biochemical and physiological changes in organisms (Gulhan et al., 2012; Talas et al., 2012). Propolis extracts, prepared with ethanol were shown to have positive effects in both animals and patients (Talas and Gulhan, 2009; Gogebakan et al., 2012). We demonstrated that the negative effects, observed as a result of cypermethrin exposure, could be reversed by adding supplementary propolis.

Many reports indicate that propolis and its constituents protect against neuronal death at least partly by the mediation of their antioxidant activity. Numerous studies have led in recent years to the idea that different propolis samples could be completely different in their chemistry and biological activity. Kasai et al. (2011) reported that Chinese propolis contains many biologically active constituents expected to be useful for improvement of the neuropathological conditions in the injured spinal cord. For example, caffeic acid phenethyl ester (CAPE), biologically active constituent in propolis, is a constituent identified to exert antiinflammatory activity and to protect the brain from ischemia-reperfusion injury (Kasai et al., 2011). The flavonoids in propolis, potentially represents antioxidative capacity in neuronal cell death. With regard to the effects of propolis on the nervous system, evidence showing that propolis and/or its components are neuroprotective against various brain insults in vivo or neuronal damage in vitro has been rapidly accumulating (references are needed). For instance, CAPE and water soluble components of propolis are neuroprotective against excitotoxic insults in ischemia/reperfusion injury. Chinese propolis may become a promising tool for wide use in the nervous system for reducing the secondary neuronal damage following primary physical injury (Bankova, 2005; Izuta et al., 2008; Kasai et al., 2011). In agreement with these results we demonstrated the direct effect of propolis on brain tissue.

Recent interests are focusing on the use of nonenzymatic antioxidants such as flavonoids in reducing the toxicity associated with pesticide-induced oxidative stress in experimental models (Kanbur et al., 2009; Alirezaei et al., 2012; Gulhan et al., 2012). Plant-based pharmaceuticals including flavonoids have been employed in the management of various diseases. They are an essential part of human diet and are present in plants that have been used for centuries in medicine. Antioxidant properties, ROS scavenging, and cell function modulation of flavonoids could account for the large part of their pharmacological activity (Narenjkar et al., 2011).

In the present study, it was determined that cypermethrin increased oxidative stress parameters in brain tissue of rainbow trout. The experimental results indicate that propolis may be beneficial in reversing adverse effects in the brain of rainbow trout exposed to cypermethrin. Chemicals used in agriculture may have negative effects on aquatic organisms and environmental monitoring reports need to be done periodically in order to limit and prevent adverse environmental contaminations.

Our results indicate that propolis has antioxidant and neuroprotective effect and can prevent brain dysfunction in fish.

Acknowledgement

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

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