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Antagonistic activity of dietary allicin against deltamethrin-induced oxidative damage in freshwater Nile tilapia; *Oreochromis niloticus*

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ABSTRACT

Allicin, the main biologically active component of garlic clove extracts, has been evaluated for its′ efficacy in preventing deltamethrin-induced oxidative damage in Nile tilapia; *Oreochromis niloticus*. Fish were fed on 2 different doses of 0.5 g and 1 g of allicin/ kg diet for 28 days. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), cholesterol, urea, uric acid, creatinine, total protein, albumin and globulin were estimated. Moreover, the level of malonaldehyde (MDA) was analyzed as a lipid peroxidation marker. In addition, reduced glutathione (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) were analyzed as antioxidant biomarkers in liver, kidney and gills. Results show that deltamethrin subacute intoxication (1.46 μg/L for 28 days) increased serum AST, ALT, ALP, cholesterol, urea, uric acid, creatinine and tissue MDA. At the same time, serum total protein and albumin as well as tissue level of GSH, GSH-Px, SOD and CAT were reduced. Allicin supplemented diets enhanced all the altered serum biochemical parameters as well as tissues′ lipid peroxidation and antioxidant biomarkers in a dose-dependent manner. The results suggest that feeding allicin can ameliorate deltamethrin-induced oxidative stress and might have some therapeutic properties to protect Nile tilapia on subacute deltamethrin toxicity.

1. Introduction

Natural products, including medicinal plants and their active constituents have played a highly significant role over the years in the discovery of new drugs for prevention and treatment of various diseases (Abdel-Daim et al., 2014; Eldahshan and Abdel-Daim, 2014; Ibrahim and Abdel Daim, 2015). They were used against diverse chemicals, drugs and xenobiotics-induced toxicity (Abdel-Daim, 2014a; Abdou and Abdel-Daim, 2014; Madkour and Abdel-Daim, 2013). Garlic *(Allium sativum)* is one of the earliest known medicinal plants. Its cloves had been used as a cure for many diseases in ancient Egypt and are mentioned in the Ebers Papyrus. When the garlic clove is crushed, the odorless compound alliin is converted to allicin, via allinase enzyme (Ankri et al., 1997). Allicin is the main biologically active component of garlic clove extracts representing about 70% of all thiosulfinates formed in crushed garlic (Calvey et al., 1994), and gives the garlic its characteristic smell (Kumar and Berwal, 1998). Pharmacologically, garlic is considered as immunomodulator, antioxidant, hypolipidemic, hypoglycemic, anticoagulant, and antimicrobial activities (Banerjee et al., 2003; Butt et al., 2009).

In fish farming, garlic has been used as a growth promoter increasing fish body gain, feed intake and feed efficiency ratio (Huang et al., 2013). In addition, it is used for enhancing the activity of non-specific defense systems and diseases′ resistance in *Oreochromis niloticus* culture (Nya and Austin, 2011) and has proven to decrease the toxicity of cadmium (Kumar et al., 2009), and lead (Shahsavani et al., 2011), and to increase resistance against *Aeromonus hydrophila* (Nya and Austin, 2009; Nya et al., 2010). It has antiparasitic activity against trichodinosis and gyrodactylosis in Nile tilapia and common carp (Abd El-Galil and Aboelhadid, 2012; Pena et al., 1988).

The synthetic pyrethroids are among the most potent and effective insecticides (El-Sayed et al., 2007). Their easy biodegradability as well as low toxicity to mammals and birds, increasing their use in agriculture (Kale et al., 1999). Pesticides applied to the land may be washed into surface waters and may kill or at least adversely influence the life of aquatic organisms (Ramwell et al., 2004). Deltamethrin (DLM) and other pyrethroids have been found to be extremely toxic to many fish species (Amin and Hashem, 2012; Cengiz et al., 2012; Ensibi et al., 2013; Haverinen and

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Vornanen, 2014; Kan et al., 2012; Kaur et al., 2011). The potential hazard to fish is not only due to its heavy use in many aquatic larvicidal programs, but also due to its relatively slow metabolism and sluggish elimination from the fish body (Bradbury and Coats, 1989b).

Pyrethroids are absorbed directly via the gills into the blood stream (Aydin et al., 2005). Among them, DLM is considered as the most potent inhibitor for fish carbonic anhydrase enzymes, causing undesirable results by disrupting acid–base regulation as well as salt transport (Richterova and Svobodova, 2012). The modifying effect of DLM on sodium and potassium channels has been demonstrated also in molluscan neurons (Kiss, 1988). Pyrethroids have been shown to be neurotoxic and lethal to fish at concentrations 10–1000 times lower than corresponding values for mammals and birds (Bradbury and Coats, 1989a).

Some reports have been studied either the effect of acute and subacute toxicity of DLM on fish (El-Sayed and Saad, 2008; El-Sayed et al., 2007; Golow and Godzi, 1994; Koprucu and Seker, 2008; Kumar et al., 1999; Velisek et al., 2006, 2007) and could prove that subacute DLM toxicity caused severe metabolic disorders and histopathological alterations that could exaggerate any diseased condition leading to serious loss of fish production. To the best of our knowledge there is lack of information on how to overcome the adverse effect of DLM using medicinal plants and their active constituents instead of chemical compounds in aquaculture. So the main objective of the present study was to evaluate the protective effects of allicin as a cheap, available and potent medicinal plant active constituent to restore the subacute DLM adverse effects in Nile tilapia; *O. niloticus*.

2. Materials and methods

2.1. Chemicals

Deltamethrin (Butox® 50 mg/ml) was purchased as a commercial product in clinical formulation from (Intervet Co., France). Pure Allicin powder was purchased from (ANHUI RUISEN BIO TECH, China). The kits used for biochemical measurements of transaminases (ALT and AST), alkaline phosphatase (ALP), cholesterol, total protein, albumin, uric acid, urea, creatinine, malondialdehyde (MDA), reduced glutathione (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) were all purchased from Biodiagnostic Co. (Dokki, Cairo, Egypt). All other chemicals used in the experiment were of analytical grade.

2.2. Experimental design and fish grouping

A total number of 100 apparently healthy male monosex Nile tilapia; *O. niloticus* were obtained from a private fish farm in EL Dakahlia Governorate, Egypt. All fish used were with an average body weight of 56 ± 5.8 g (Mean $\pm$ SE) and from 10–12 cm length. Fish were transported alive to the laboratory of Department of Fish Diseases and Management, Faculty of Veterinary Medicine, El Mansoura University, Mansoura, Egypt. Fish were separated into 5 groups (20 fish/ group), and were kept in 10 glass aquaria (120 effective liter each, 10 fish/ glass aquarium) either for two weeks during acclimatization or during the experimental period (28 days). The water parameters were maintained according to the optimum requirement for tilapia (temperature, 25 ± 1 °C and the photoperiod was 12 h, pH 7.2, dissolved oxygen 6.9 mg/L).

Experimental design and fish handling were approved by the Research Ethical Committee of the Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt (the approval no. 20147) as well as by Research Ethical Committee of the Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt. All groups either were fed on basal diet or allicin supplemented diet as for 28 days. The 1st group, served as a control, and fed basal diet, 2nd group was fed allicin supplemented diet at 1 g/kg diet concentration. The 3rd group was fed a basal diet and exposed to DLM at concentration (1.46 μg/l) in water for 28 consecutive days, and this concentration represented as 1/10 of the LC50 as measured by El-Sayed et al. (2007). Moreover the concentration dose and duration were chosen according to Abdelkhalek et al. (2014), El-Sayed and Saad (2008). The 4th and 5th groups were fed allicin supplemented diet at concentration of 0.5 g and 1 g/kg diet respectively (Abdelhamid et al., 2013), and exposed to DLM at the same concentration and duration of that of 3rd group. A basal diet was formulated to contain 31.78% crude protein, 7.15% lipids and 8.14% ash (Supplementary Materials, Table 1). The basal and allicin supplemented diets were prepared biweekly and stored in a refrigerator (4 °C) for daily use. The dead fish were removed as soon as possible to avoid possible water deterioration, and the mortality rate was recorded during the experimental period. The clinical symptoms of fish exposed to DLM were observed daily compared to the control group.

2.3. Serum biochemical analysis

At the end of the experimental period blood was collected from fish of each group through caudal vessels, and transferred to clean dry centrifuge tubes then left for 2 h for clotting and centrifuged at 1200 g for 15 min at 4 °C followed by serum separation.

Freshly separated sera were used for estimation of serum hepatorenal injury biomarkers according to manufacturer protocol. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were evaluated using a colorimetric method according to Reitman and Frankel (1957), Alkaline phosphatase (ALP) using an enzymatic colorimetric method according to Tietz et al. (1983). The enzyme activity was expressed as units/liter computed directly from the absorbance values. Serum total protein was measured according to Lowry et al. (1951) and albumin according to Young et al. (2001). Cholesterol was measured according to Allain et al. (1974), Richmond (1973). For analysis of renal products, creatinine was determined according to Larsen (1972), urea according to Coulombe and Favreau (1963) and uric acid according to Whitehead et al. (1991).

2.4. Evaluation of tissue total protein, lipid peroxidation and antioxidant biomarkers

Liver, kidneys and gills′ tissues of fish from each group were rapidly removed, cleaned from any extraneous materials and immediately perfused with cold saline. The tissues were homogenized in cold phosphate buffer saline (0.1 M pH 7.4) then the homogenate was filtered and centrifuged at 1800 g for 20 min at 4 °C using a high speed cooling centrifuge (Type 3K-30, Sigma, Osterode-am-Harz, Germany) to separate the nuclear debris. The supernatant was aspirated and stored in −80 °C until use for biochemical analysis.

Total protein level in hepatic, renal and gills′ tissues were evaluated according to Lowry et al. (1951) using bovine γ-globulin as standard. The results were expressed as milligrams of protein per gram of tissue. Lipid peroxidation was evaluated by measurement of MDA content in hepatic, renal and gill tissues according to spectrophotometric method of Mihara and Uchiyama (1978). Oxidative stress was assessed by evaluation of the enzymatic antioxidant biomarker; superoxide dismutase (SOD) according to Nishikimi et al. (1972), catalase (CAT) according to Aebi (1984) and glutathione peroxidase (GSH-Px) according to Paglia and Valentine (1967). The non-enzymatic antioxidant marker;