Journal of Advanced Research (2014) 5, 671–684

Cairo University

Journal of Advanced Research

ORIGINAL ARTICLE

Modulation of genotoxicity and endocrine disruptive effects of malathion by dietary honeybee pollen and propolis in Nile tilapia (*Oreochromis niloticus*)

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A R T I C L E I N F O

Article history:
Received 23 June 2013
Received in revised form 28 October 2013
Accepted 30 October 2013
Available online 8 November 2013

Keywords:
Genotoxicity
Malathion
Nile tilapia
Pollen
Propolis

A B S T R A C T

The present study aimed at verifying the usefulness of dietary 2.5% bee-pollen (BP) or propolis (PROP) to overcome the genotoxic and endocrine disruptive effects of malathion polluted water in *Oreochromis niloticus* (*O. niloticus*). The acute toxicity test was conducted in *O. niloticus* in various concentrations (0–8 ppm); mortality rate was assessed daily for 96 h. The 96 h-LC_{50} was 5 ppm and therefore 1/5 of the median lethal concentration (1 ppm) was used for chronic toxicity assessment. In experiment (1), fish ($n = 8$/group) were kept on a diet (BP/PROP or without additive (control)) and exposed daily to malathion in water at concentration of 5 ppm for 96 h "acute toxicity experiment". Protective efficiency against the malathion was verified through chromosomal aberrations (CA), micronucleus (MN) and DNA-fragmentation assessment. Survival rate in control, BP and PROP groups was 37.5%, 50.0% and 100.0%, respectively. Fish in BP and PROP groups showed a significant ($P < 0.05$) reduction in the frequency of CA (57.14% and 40.66%), MN (53.13% and 40.63%) and DNA-fragmentation (53.08% and 30.00%). In experiment (2), fish (10 males and 5 females/group) were kept on a diet with/without BP for 21 days before malathion-exposure in water at concentration of 0 ppm (control) or 1 ppm (Exposed) for further 10 days "chronic toxicity experiment". BP significantly ($P < 0.05$) reduced CA (86.33%), MN (82.22%) and DNA-fragmentation (93.11%), prolonged the sperm motility when exposed to 0.01 ppm of pollutant *in vitro* and increased the estradiol

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Peer review under responsibility of Cairo University.

ELSEVIER Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jare.2013.10.004

level in females comparing to control. In conclusion, BP can be used as a feed additive for fish prone to be raised in integrated fish farms or cage culture due to its potency to chemo-protect against genotoxicity and sperm-teratogenicity persuaded by malathion-exposure.

Introduction

Fish are being used as useful genetic models for evaluation of pollution in aquatic ecosystems. Fish as bio-indicators of pollutant effects are very sensitive to the changes in their environment and play significant roles in assessing potential risk associated with contaminations of new chemicals in aquatic environment [1]. The sub-lethal toxicity of pesticides decreases plankton abundance and water quality in fish ponds [2]. Moreover, pesticides have been noticed to interfere with fish health and reproduction [3].

Malathion (*O, O-dimethyl phosphorodithioate of diethyl mercaptosuccinate*) is a colorless to amber liquid with a skunk- or garlic-like odor [4]. It is a broad-spectrum insecticide widely used to control a variety of outdoor insects in both agricultural and residential settings [5] because of its effectiveness and shorter duration in the aquatic environment. In soil, malathion is not considered a persistent pesticide (log Kow 2.89, half-life 1–10 days) [6]. In water, the half-life of malathion has been estimated as 1.65 days at pH 8.16 and 17.4 days at pH 6.0 [7]. The degradation rate of malathion has been found to be 0.017 ppm/h [8]. Degradation of malathion in river, ground and seawater (avg. $t1/2 = 4.7$ d) is controlled by an elimination reaction, photolysis and biodegradation [9]. Malathion tends to be relatively non-mobile in aqueous environment because it absorbs into sediments [10] and once adsorbed it is typically degraded within 3 days [11].

When malathion is introduced into the environment, it may cause serious intimidation to the aquatic organisms as well as severe metabolic disturbances in non-target species like fish and fresh-water mussels [6]. Sub-lethal doses of malathion in Nile tilapia lead to a decrease in fish growth rate and deterioration in their physiological condition. Higher concentrations of this pesticide lower the production and profitability of freshwater fish farms [2]. *O. niloticus* exposed to malathion in feed (0.17 mg/kg) for long term (120 days) exhibits an alternation of sex steroid hormones, degenerative changes in gonads and poor milt quality [12].

Diverse methods have been adopted for evaluating the potential toxicological effects of aquatic pollutants. The incidence of micronuclei in fish peripheral erythrocytes [7], comet assay [13] as well as mitotic chromosomes of the head kidney [14] have been used as an imperative tool for monitoring genotoxicity in aquatic environments.

The frequency of micronucleus (MN) in the peripheral blood erythrocytes is one of the best established *in vivo* cytogenetic assays in the field of genetic toxicology, providing a convenient and reliable index of both chromosome breakage and chromosome loss [15]. Therefore, MN is recommended to be conducted as a part of the monitoring protocols in aquatic toxicological assessment programs [16].

Teleost head kidney (HK) has been considered as a haemopoietic organ similar to the bone marrow of higher vertebrates characterized by high proportion of actively dividing cells [17]. Standard procedures for mitotic chromosomal preparation from the HK tissue have been used to gain information about the nature and extent of the damage that may be produced by *in vivo* treatments [18]. The mitotic chromosomes from the HK of the fish *Tilapia niloticus* have been studied with an initiative to gain information about the nature and extent of the damage that may be produced by *in vivo* treatments [14].

Liver is the major site of xenobiotic accumulation and biotransformation, analyses of initial molecular lesions elicited by pollutants in this organ gives early-warning and sensitive indicator of chemical induced carcinogenic lesions [19]. So, it was reliable to use the liver cells as an indicator for the genotoxic effect of malathion using comet assay.

Nowadays, a great concern is directed toward the use of natural products for improving fish health status, and consequently increasing the resistance to stressors including pollutants. Flavonoids are naturally produced in plants and stored in different forms such as propolis [20]. The biological activities of propolis depend on the presence of flavonoids, aromatic acids, diterpenic acids and phenolic compounds which have important pharmacological properties. Propolis is an alternative dietary antibiotic [21] that is effective against a variety of bacteria [22], viruses [23] and fungi [24], and is beneficial for improving the performance and immunity [25].

Bee pollen is considered as one of nature's most completely nourishing foods since it contains essential substances such as carbohydrates, proteins, amino acids, lipids, vitamins, mineral substances and trace elements [26]. The main bioactive compounds reported from bee pollen are phenolic compounds and specifically quercetin, kaempferol, caffeic acid [27] and naringenin [28]. Globally bee pollen has been reported to provide a diverse array of bioactivities, such as anti-proliferative, anti-allergic, antibiotic, anti-diarrheic and antioxidant activities [29,30].

The present work aimed at verifying the protective effect of honeybee products (propolis and pollen) supplemented in the feed of Nile tilapia (*Oreochromis niloticus*) against the genotoxic and reproduction disruptive effects of acute and chronic exposure to malathion polluted water.

Material and methods

Fish

Oreochromis niloticus (*O. niloticus*) was obtained from a private fish farm in the Kafr El Sheikh Governorate, Egypt. They were stocked in fiberglass 750 L-tanks ($n = 50$ of both sex/tank) supplied with continuous aerated dechlorinated water (26 ± 2 °C) at the Faculty of Veterinary Medicine, Benha University, Egypt. Fish were fed with commercial pelleted diet (JOE Trade, Cairo, Egypt) at 5% of their body weight daily and kept for two months until they reached a mean weight of 63 g. The chemical compositions and proximate analysis of the ingredients used in the commercial diet (crude protein 30%) are shown in Table 1. Uneaten food particles and excreta were removed by the daily siphoning with exchanging of about