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Inhibition of cadmium- induced genotoxicity and histopathological changes in Nile tilapia fish by Egyptian and Tunisian montmorillonite clay

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ABSTRACT

Cadmium (Cd) is an important inorganic toxicant widely distributed in the environment because of its various industrial uses. The aims of the current study were to investigate the efficacy of purified Egyptian and Tunisian montmorillonite clays (EMC and TMC) to inhibit genotoxicity and histological alterations induced by cadmium chloride ($CdCl_2$) utilizing the Nile tilapia fish as an in vivo model. Chromosomal aberrations (CAs), micronucleus (MN) frequencies and DNA fingerprinting profile were genotoxic end points and histopathological changes that were used in this investigation. Six groups of fish were treated for 2 weeks and included control group, $CdCl_2$-treated group and groups treated with EMC or TMC alone or in combination with $CdCl_2$.

The present results revealed that, treatment of fish with $CdCl_2$ exhibited significant increased in the number of micronucleated erythrocytes (MnRBCs), frequency of CAs and instability of genomic DNA. Treatment of EMC and TMC in combination with $CdCl_2$ significantly reduced the frequency of MnRBCs by the percentage of 53.28% and 60.77% and the frequency of CAs by 43.91% and 52.17% respectively. As well as, normalized DNA fingerprinting profile and significantly improved histopathological picture induced by Cadmium treatment. It is worth mention that both clays have the ability to tightly bind $CdCl_2$ and decreased its cytotoxicity and genotoxicity; however, Tunisian clay was more efficient in binding with the $CdCl_2$ than Egyptian clay.

1. Introduction

The contamination of food and feedstuff with heavy metals represents many problems for human and animal's health, thereby continuously attracting worldwide attention (Satarug et al., 2003; FAO/WHO, 2003). Heavy metal contamination in the terrestrial as well as the aquatic environment is a worldwide problem of increasing magnitude. Heavy metals can affect aquatic organisms through water, sediment or food chain (Zyadah, 1995).

Cadmium (Cd) is an important inorganic toxicant widely distributed in the environment because of its various industrial uses and it is a nonessential element to all living organisms (Randi et al., 1996; Besirovic et al., 2010). Also contamination of groundwater from smelting and industrial uses as well as the use of sewage sludge as a food-crop fertilizer. Cadmium was known by their bioaccumulation and caused harmful effects in different levels of the trophic chain (Stoeppler, 1991). It is well known as carcinogen (Banerjee and Flores-Rozas, 2005), teratogenic (Hovland et al., 2000), caused sterility (Bench et al., 1999), nephrotoxic (Ahmed and Abdel-Wahhab, 2000; Cai et al., 2001), hepatotoxic (Horiguchi et al., 2000; Ahmed and Abdel-Wahhab, 2000), genotoxic and apoptotic (Kim et al., 2005; Mondal et al., 2005) and also inducing pancreatic activity changes (Shimada et al., 2000). For this fact, excessive exposure to Cd results in diseases and occasionally death (Othumpangat et al., 2005).

Cadmium that enters aquatic environments accumulates within the bodies of aquatic organisms (Habib and Samah 2013). The growth, osmoregulation and reproduction of fish are affected by exposure to this metal (Kim et al., 2004). Cd obstructs numerous reproductive processes in fish such as sexual maturation, spermatogenesis, fertilization success and development of the embryonic and post embryonic stages (Jezierska and Witeska, 2001; Dietrich et al., 2011). It is also causes DNA single-strand breaks, DNA–protein crosslinks, chromosome aberrations (CAs), and apoptosis (Hovland et al., 2000). Previously, Muley et al. (2000), found significant alterations in the DNA and RNA contents

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in gills, liver and brain of the common carp, Cyprinus carpio exposed to cadmium chloride and lead acetate. Also Bais and Lokhande (2014) found reduction of DNA in Ophiocephalus straatus fish.

Many of common clays and zeolitic minerals may be non-selective in their action and may pose significant hidden risks due to interaction with nutrients and heavy metals. Several reports have indicated that the phyllosilicate montmorillonite clay, which is currently used as an anti-caking agent for animal feed borne metals uptake, may remove heavy metals from aqueous solutions (Subramanian and Gupta 2006; Da Fonseca et al., 2006). In the aquatic environment, Montmorillonite Clay binds toxins and heavy metals to a degree that far outperforms charcoal and other filter products, plus it supports beneficial bacteria also, the Montmorillonite Clay has a protective effect against some mycotoxins (sterigmatocystin and aflatoxin) and could be used to protect cultured tilapia from such toxins (Mahrous et al., 2006; Hassan et al., 2010). Scientifically speaking, most bentonite clay minerals have peculiar adsorption arising from their layered structure, charged layers and active edges. The layered structure provides inter-layer space to host guest molecules and ions. Charged layers and "broken edge" sites attract varieties with opposite charges through Van der Waals force. Such features allow clay minerals to be used as adsorbents for the removal of heavy metal ions from water. Calcium Montmorillonite Clay can also be effective adsorbents and absorbents of oily pollutants and animal waste (Mckinnon, 2013).

Therefore, the application of the purified montmorillonite clay collected from Tunisian and Egypt environments as adsorbents for remediation of Cd toxicity is of great national and international interest. The aim of the present study was to evaluate the protective effect of Egyptian and Tunisian clay on the chromosomal aberrations (CAs), micronuclei (MNs), DNA and histo-pathological changes in tilapia exposed to cadmium.

2. Materials and methods

2.1. Chemicals

2.1.1. Cadmium chloride

Cadmium chloride was purchased in a pure standard solution in concentration 1000 mg /L from Merck, 64271 Darmstadt (Germany).

2.1.2. Tunisian montmorillonite clay

The results of the chemical analysis of TM revealed that was composed of 52.61% SiO_2, 10.11% Al_2O_3, 5.10% Fe_2O_3, 0.44% TiO_2, 1.05% MgO, 5.75% CaO, 2.22% Na_2O, 2.30% K_2O, 20.19% FL, and 0.19% SO_3 based on the dry weight.

2.1.3. Egyptian montmorillonite clay

It was kindly supplied from the Ceramic Department, National Research Center, Dokki, Egypt. The results of the chemical analysis of EM revealed that was composed of CaO 0.99%, SiO_2 43.90%, Al2O3 18.64%, Fe_2O_3 9.50%, MgO 2.04%, SO_3 0.07%, $K_2O_3$1.08%, Na_2O 2.10% and Cl^- 4.21%.

2.2. Fish

Sixty apparently healthy, two-month-old Nile tilapia fish *(Oreochromis niloticus)* with an average body weight of 90 ± 10 g were purchased from El-Nobarya Fish Farm (El-Nobarya, Egypt) and transported alive in a large plastic water container supplied with battery aerators as a source of air. During transportation fishes were treated with lidocaine (5 mg/L) to reduce stress. Fish were maintained on a standard fish diet at the Animal House, Faculty of Veterinary Medicine, Cairo University (Giza, Egypt). Feeding was done once daily using a pelleted diet (32% protein ration) at rate of 3% of the fish body weight. The water in aquaria was changed daily to avoid metabolite accumulations in glass aquaria (static system). After an acclimation period of one week, the fish were divided into six experimental groups (10 fish/group) and each group was placed in a fully prepared aquarium containing de-chlorinated tap water, the average water temperature was 20 ± 3.7 °C and the pH was in the range 7.17–8.19.

2.3. Experimental design

Fish within different treatment groups were treated for 2 weeks as follows:

Group 1, untreated control group fed on the standard diet; **Group 2**, treated orally with Egyptian Montmorillonite clay (EMC) alone at level (400 mg kg^{-1} BW); **Group 3**, treated orally with Tunisian Montmorillonite clay (TMC) alone at level (400 mg kg^{-1} BW); **Group 4**, treated orally with $CdCl_2$ (2.5 mg kg^{-1} BW) in water; **Group 5**, treated orally with $CdCl_2$ (2.5 mg kg^{-1} BW) simultaneously with EMC (400 mg kg^{-1} BW) and **Group 6**, treated orally with $CdCl_2$ (2.5 mg kg^{-1} BW) simultaneously with TMC (400 mg kg^{-1} BW).

2.4. Cytogenetical investigations

2.4.1. Micronucleus test

A drop of blood from the gills was mixed with a drop of fetal calf serum and smeared directly on slide then air dried, fixed in absolute methanol for 5 min and stained with 5% Giemsa for 7 min. Two thousand of red blood cells per fish were analyzed for the frequency of MN erythrocytes. The diameter of the micronucleus (MN) was less than one-third of the main nucleus, separated from or marginally overlapped with main nucleus and had similar staining as the main nucleus. The number of micronucleated erythrocytes (Mn-RBCs) was expressed per 2000 erythrocytes (De Flora et al., 1993).

2.4.2. Chromosomal preparation

Chromosomal preparation of kidney tissues was carried out according to the method described by (Al-Sabti, 1986) with some modification. In brief the anterior kidney from each fish was excised and cut into fine particles in 5–7 ml of RBMI medium and 0.2 ml of 0.05 colchicine was added to each tube in vitro. Cultures were incubated at 37-38 °C for 1 h then the cells were centrifuged at 1000 rpm for 10 min and resuspended in pre warmed (37 °C) hypotonic solution (KCl 0.5%) for 30 min at 37 °C. The sample were centrifuged and fixed; the slides were produced by the conventional method and stained with Giemsa stain. Chromosome analysis was carried out in 100 metaphase spreads for each fish.

2.5. Molecular genetics analysis

2.5.1. DNA extraction

DNA was extracted from the liver fish according to the method described by Sambrook et al. (1989). The genomic DNA was isolated using phenol/chloroform extraction and ethanol precipitation method with minor modifications.

2.5.2. Random amplified polymorphism DNA (RAPD- PCR)

RAPD-PCR was carried out with the pooled and the individual genomic DNA samples. A total of six random decamer primers of arbitrary sequences with 60-70 GC% content were used as listed in Table (1). The amplification conditions and PCR mixture were set according to Williams et al. (1990) and Plotsky et al. (1995),