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Effectiveness of activated carbon and Egyptian montmorillonite in the protection against deoxynivalenol-induced cytotoxicity and genotoxicity in rats

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ABSTRACT

This study was conducted to prepare and characterize activated carbon (AC) and to evaluate its protective effect against deoxynivalenol (DON) toxicity in rats compared to Egyptian montmorillonite (EM). AC was prepared using a single-step chemical activation with phosphoric acid (H_3PO_4). The resulted AC has a high surface area and a high total pore volume. Male Sprague–Dawley rats were divided into 6 groups (n = 10) and treated for 3 weeks as follow: the control group, the groups fed AC or EM-supplemented diet (0.5% w/w), the group treated orally with DON (5 mg/kg b.w.) and the groups fed AC or EM-supplemented diet and treated with DON. Blood and liver samples were collected for different analyses. Treatment with DON increased liver function enzymes, lipid peroxidation, tumor necrosis factor α, DNA fragmentation, decreased hepatic glutathione content, up regulating mRNA Fas and TNF-α genes expression and increased micronucleated polychromatic erythrocytes and normochromatic erythrocytes in bone marrow. Co-treatment of DON plus AC or EM succeeded to normalize the levels of the biochemical parameters, reduced the cytotoxicity of bone marrow and ameliorated the hepatic genotoxicity. Moreover, AC was more effective than EM and has a high affinity to adsorb DON and to reduce its cytotoxicity and genotoxicity.

1. Introduction

Deoxynivalenol (DON, vomitoxin) is a type B trichothecene produced by *Fusarium graminearum* and *Fusarium culmorum* that contaminate grains such as wheat, maize, barley, and oats (Ennouari et al., 2013; Santos et al., 2013). DON is mainly produced in the field conditions or during storage (Ma and Guo, 2008). The presence of DON in human food resulted in serious health concerns including anorexia and vomiting and disrupts cell signaling, differentiation, growth and macromolecular synthesis leading to gastrointestinal homeostasis, disturbances in neuroendocrine function and immunity (Pestka and Smolinski, 2005). It was reported that DON impairs growth in laboratory animals (Pinton et al., 2008). Pestka (2008) suggested that DON can be either immunosuppressive or immunostimulatory depending on the dose and frequency of exposure; since it interacts with peptidyl transferase in the 60S ribosomal subunit and triggers ribotoxic stress. Moreover, Rizzo et al. (1994) suggested that exposure to DON resulted in the production of free radicals which considered one of the mechanisms that cause damage to the cellular membrane and to DNA. Consequently, oxidative stress is regarded as an important factor in DON-induced toxicity. In a recent report, Yang et al. (2014) indicated that DON decreases cell viability, causes damage to the cellular membrane, chromosomes, or DNA, induce lipid peroxidation and raises the levels of 8-OHdG and reactive oxygen species (ROS) in human peripheral blood lymphocytes. It was also reported that DON inhibit protein, DNA and RNA synthesis (Audenaert et al., 2013). Generally, DON seems to be one of the most important trichothecenes mycotoxins in cereal contamination with significant health and economic concern (EC, 2003).

The FAO/WHO (2001) reported that elimination of DON from contaminated feedstuffs is a main issue and few strategies can be

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adopted for limiting adverse effects. Previously, Abdel-Wahhab et al. (1999, 2005) reported that the most interesting approaches for the decontamination of mycotoxins in feedstuffs is the use of adsorbent materials that may bind mycotoxins in the gastrointestinal tract, thus reducing the extent of their absorption and systemic toxicity. Several products have been tested for their effectiveness to detoxify DON in contaminated feed, but the results continue to be unsatisfactory. Particularly, most of the adsorbing agents bind DON weakly *in vitro* and are ineffective *in vivo* (Dänicke, 2002). Moreover, the chemical binding agents such as polyvinylpyrrolidone or ammonium carbonate had no effect on the reduction of DON toxicity in pigs (Friend et al., 1984). The addition of either bentonite or hydrated sodium calcium aluminosilicate to DON-contaminated corn was not effective in overcoming the depressed performance effects of DON in pigs (Ramos et al., 1996; Visconti, 1998). Although activated carbons (AC) have been shown to adsorb DON from aqueous solutions (Galvano et al., 1998), the *in vitro* binding of mycotoxins by AC or other adsorbent materials cannot simply be extrapolated to *in vivo* efficacy (Visconti, 1998). The *in vivo* experiments may lead to large individual differences and inconsistent results because feed intake may be influenced by several factors including age, body weight, sex, feeding period, feeding system and the genetic heterogeneity (Eriksen, 2003). The aims of the current study were to prepare and characterize AC from the date stones as agriculture waste and to evaluate its efficiency against DON-induced oxidative stress and unregulated the apoptotic genes expression compared to the Egyptian montmorillonite using rats as animal model.

2. Materials and methods

2.1. Materials

Egyptian montmorillonite (EM) was obtained from the Egyptian Bentonite and Derivatives Co. (Alexandria, Egypt). Date stones were obtained from the Egyptian date Co. (Cairo, Egypt).

2.2. Chemical, kits and reagents

DON and Biozol reagent were purchased from Sigma Chemical Co. (St. Louis, MO, USA), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) kits were purchased from Spectrum-diagnostics Co. (Cairo, Egypt). Alkaline phosphatase (ALP) was purchased from Biodiagnostic Co. (Giza, Egypt). Reduced Glutathione (GSH), lipid peroxidation (MDA) and Tumor necrosis factor α (TNF-α) enzyme-linked immunosorbent assay (ELISA) kits was purchased from Biosource Co. (Camarillo, CA, USA). Taq polymerase was purchased from PE Applied Biosystems (Foster City, CA, USA). Superscript™ II reverse transcriptase and oligonucleotide primers were purchased from Life Technologies (Carlsbad, CA, USA). Phosphoric acid (85%) was purchased from Merck (Darmstadt, Germany). All other chemicals used throughout the experiments were of the highest analytical grade available.

2.3. Preparation of activated carbon (AC)

Date stones were used as a precursor for the production of AC using a single-step chemical activation with H_3PO_4 under its own evolved gases during pyrolysis according to El-Kady et al. (2013). Briefly, one hundred grams of crushed date stones (500–850 μm) were soaked in phosphoric acid (85 wt. %) at ratio of 1:3 (w/w) and slightly agitated to ensure penetration of the acid throughout the carbonaceous raw materials. The mixture was heated to 100 °C for 2 h and left overnight at room temperature. The impregnated mixture was activated in muffle furnace at 500 °C for 2 h at a rate of 5 °C/min. The acid was removed by washing with distilled water to pH 6.8. The carbon product was dried at 110 °C for 24 h in an electric oven.

2.4. Characterization of AC

The carbon, hydrogen, nitrogen and sulfur contents of AC were determined using a CHNS elemental analyzer (Thermo Electron Flash EA 1112). The specific surface area and pore structural parameters were determined from the adsorption–desorption isotherm of nitrogen at 77 K. However, the S_{BET} was calculated by Brunauer et al. (1938) (BET) equation and the pore size distribution was calculated on the basis of desorption data following the Barrett–Joyner–Halenda (BJH) method. The surface topography of the AC was carried out using scanning electron microscope (SEM).

2.5. Experimental animals

Three-month old healthy male Sprague–Dawley rats (100–150 g each) were purchased from Animal House Colony, National Research Centre, Dokki, Cairo, Egypt. Rats were maintained on special powdered feed (protein: 160.4; fat: 36.3; fiber: 41 g/kg, namely 12.1 MJ of metabolized energy) containing no inorganic sorbents purchased from Meladco Feed Co. (Aubor City, Cairo, Egypt) and housed in filter-top polycarbonate cages in a room free from any source of chemical contamination, artificially illuminated (12 h dark/light cycle) and thermally controlled (25 ± 1 °C) at the Animal House Lab., National Research Centre, Dokki, Cairo, Egypt. All rats were received humane care in compliance with the guidelines of the Animal Care and Use Committee of the National Research Center, Dokki, Cairo, Egypt and the National Institutes of Health (NIH publication 86–23 revised 1985).

2.6. Experimental design

Two types of diets were prepared using the powdered feed which were supplemented with the two sorbents: Diet No. 1: basal feed +0.5% AC; Diet No. 2: basal feed +0.5% EM. The rats were distributed into six treatment groups (10 rats/group) and treated for 3 weeks as follows: group 1, untreated control; group 2, rats fed diet 1, group 3, rats fed diet 2; group 4, rats treated orally with DON (5 mg/kg b.w.) in corn oil; group 5, rats fed diet 1 and treated with DON and group 6, rats fed diet 2 and treated with DON. Treatment groups (AC or EM) alone and in combination with DON, were exposed to sorbents in the feed as well as by gavage. Untreated and DON, control rats were fed only the basal diet without any sorbent. Rats maintained on the diets containing sorbents (AC or EM) were gavaged with corn oil in combination with an amount of the respective sorbent equivalent to 0.5% of the estimated maximum daily intake of feed. Rats receiving DON were gavaged with the toxin in the corn oil alone or concomitantly with a similar quantity of the respective sorbent. The sorbent was mixed with the toxin in the corn oil just prior to dosing to maintain sufficient sorbent availability to the toxin. Dosing volumes were 0.5 ml/kg b.w. in all treatment groups. At the end of the treatment period (i.e. day 21), all animals were fasted for 12 h, then blood samples were collected from the retro-orbital venous plexus under diethyl ether anesthesia. Sera were separated using cooling centrifugation and stored at −20 °C until analysis. The sera were used for the determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) according to the kits instructions. After the collections of blood samples, rats were sacrificed and samples of femur bone marrow were collected for the micronucleus assay. Samples of the liver of each animal were dissected and kept at −80 °C for the determination of different