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Evaluation of the bioactive extract of actinomyces isolated from the Egyptian environment against aflatoxin B_1-induce cytotoxicity, genotoxicity and oxidative stress in the liver of rats

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ABSTRACT

This study aimed to determine the bioactive compounds of actinomyces (ACT) isolated from the Egyptian environment (D-EGY) and to evaluate their protective activity against AFB_1 in female Sprague-Dawley rats. Six groups of animals were treated orally for 3 weeks included: C, the control group, T1, AFB_1-treated group (80 μg/kg b.w), T2 and T3, the groups received ACT extract at low (25 mg/kg b.w) or high (50 mg/kg b.w) doses, T4 and T5, the groups received AFB_1 plus the low or high dose of ACT extract. Blood, bone marrow and tissue samples were collected for different analyses and histological examination. The results revealed the identification of 40 components, representing 99.98%. Treatment with AFB_1 disturbs liver function parameters, oxidative stress markers, antioxidant gene expressions, DNA fragmentation and induced severe histological changes. ACT extract at the low or high doses did not induce significant changes in all the tested parameters or histological picture of the liver. Moreover, ACT extract succeeded to induce a significant protection against the toxicity of AFB_1. It could be concluded that the bioactive compounds in ACT are promise candidate for the development of food additive or drugs for the protection and treatment of liver disorders in the endemic area.

1. Introduction

Aflatoxins are the toxins elaborated by the fungi *Aspergillus flavus* and *A. parasiticus*, contaminate multiple staple foods, including cereals, groundnuts and tree nuts (Idris et al., 2010; Iqbal et al., 2012, 2013). It has been estimated that 55 billion of people all over the world may exposed to uncontrolled aflatoxins in the diet (Strosnider et al., 2006; Kensler et al., 2011). Human exposed to AFs by the indirect source when nourished products of animals that have been ingested contaminated feed. AFs being highly lipophilic are quickly absorbed via the alimentary canal. Moreover, they also can enter the bloodstream directly by the inhalation (Bbosa et al., 2013). AFs especially aflatoxin B_1 (AFB_1) are well known potential carcinogen and was categorized as the group I human carcinogen (IARC, 1993). AFB_1 work synergistically with some human health factors to the development of cancer including hepatitis B virus infection, nutritional status, sex, age and the amount of AFs exposure (Qureshi et al., 2014; Wild and Montesano, 2009). AFB_1 induce a variety of biological activities like acute toxicity, teratogenicity, mutagenicity, growth stunting, immunosuppression, genotoxicity, produce membrane damage through increased the peroxidation of lipid and the generation of free radicals (Abbès et al., 2010; Abdel-Wahhab et al., 2012; Mary et al., 2012). Furthermore, AFB_1 was found to suppress $CD14^-$mediated production of nitric oxide in murine peritoneal macrophages (Moon and Pyo, 2000) and increase the prevalence of hepatocellular carcinoma (HCC) in humans (Abdel-Wahhab et al., 2012; Sun et al., 2011). Moreover, the carcinogenicity of AFB_1 is associated with the alteration of several p53-target genes expression and mutations, mainly the p53 codon 249 hotspot mutation (Josse et al., 2012).

Natural products are used for the treatment of many diseases. Plant-derived compounds are the most commonly utilized agents

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in alternative medicine. However, a number of novel therapeutic compounds are mainly derived from microbs (Cragg and Newman, 2013). Microorganisms are a rich source of diverse bioactive secondary metabolites and produced the most important products for pharmaceutical industries (Dasari et al., 2012). Actinomycetes are prokaryotic filamentous bacteria, Gram-positive, saprophytic that belong to the phylum Actinobacteria (Chaudhary et al., 2013; Barka et al., 2016) and were classified as transitional forms between bacteria and fungi (Barka et al., 2016).

They are extensively dispersed in water, soil and represent a significant ecological in the soil nutrient cycling for colonizing plants and have DNA with high GC content (69–73%) (Bizuye et al., 2013). Many antimicrobial compounds have been produced from various types of actinomycetes. *Streptomyces* spp are the dominant genera of the actinomyces (ACT) which have the largest number of species and varieties occurred in nature (Golinska et al., 2015; Lertcanawanichakul et al., 2015; Zhao et al., 2011). The bioactive secondary metabolites that produced by actinomycetes are playing an extensive role in medical and pharmaceutical industry (Gayathri and Muralikrishnan, 2013; Janardhan et al., 2014; Singh and Dubey, 2015). Many of these compounds were isolated and well characterized and have been developed into drugs for treatment of different diseases in human, animals and the agriculture sectors (Balachandran et al., 2012; Bettoli et al., 2016; Momose and Kawada, 2015). Most the known antibiotics are produced by actinomycetes (Berdy, 2005; Mullowney et al., 2015). Novel secondary metabolites showed a variety of biological activities included anti-infective, antiviral, antibacterial, antifungal, anti-parasitic, anti-cancer, antitumor, cytotoxic, antidiabetic, immunosuppressive (Antoszczak et al., 2014; Arumugam , 2012; Dasari et al., 2012; Kekuda et al., 2010; Subathra et al., 2013) and alleviated pulmonary tract fibrosis (Koh et al., 2014; Li et al., 2006). The aim of the current study was to evaluate the possible protective activity of the bioactive extract of *Streptomyces* isolated from the Egyptian environment (D-EGY) against oxidative stress, cytotoxicity and genotoxicity of AFB_1 in rats.

2. Materials and methods

2.1. Chemicals and kits

AFB_1 standards, Sodium tripolyphosphate (TPP) and RevertAid™ H Minus First Strand cDNA Synthesis Kits were purchased from Sigma Chemical Co. (St. Luis, Mo, USA). ™DNase and removal reagents kit was purchased from Promega, Co. (Madison, WI, USA). Transaminase (ALT, AST), cholesterol (Cho), triglycerides (TriG), high density lipoprotein (HDL), protein (P), urea, uric acid and createnine were purchased from FAR Diagnostics Co. (Via Fermi, Italy), catalase (CAT), nitric oxide (NO), albumin (Alb), glutathione peroxidase (GPx), superoxide dismutase (SOD) kits were obtained from Eagle diagnostics (Dallas, TX, USA). Alpha Feto protein (AFP), carceno embrionic antigen (CEA) and interleukin 6 (IL-6) kits were purchased from Immunospec (Canoga Park, CA, USA). TRIZOL reagent was purchased from Invitrogen™ (Carlsbad, CA, USA). All other chemicals used throughout the experiments were of the highest analytical grade available.

2.2. Actinomycin isolation

A total of 50 g of dried soil samples (collected from agricultural soil at Dakahlia governorate, Egypt) was suspended separately in 200 ml of sterile distilled water and shaken on a reciprocal shaker for 2 h (Atta, 2015). The isolation of actinomycetes was carried out using starch-nitrate agar (SNA) consisting of 20 g/l starch, 1 g/L KNO_3, 0.5 g/l K_2HPO_4, 0.5 g/l $MgSO_4.7H_2O$, 0.5 g/l NaCl, 0.01 g/l $FeSO_4$, 15 g/l agar and supplemented with nystatin at a concentration of 50 μg/ml as an antifungal and nalidixic acid at a concentration of 20 μg/ml as an antibacterial (Sineva and Terekhova, 2015). The shaken suspension of soil was diluted using serial dilution procedure (10^{-1} to 10^{-6}) and aliquots (100 μl) from the different dilutions were used to inoculate the medium plates. The plates were incubated at 30 °C for two weeks. The growing colonies were sub-cultured and transferred to slants without the antibiotic substance and kept at 4 °C until use (Das et al., 2008 Mincer et al., 2002). These actinomycetes strains were maintained on SNA and SCA medium and incubated for 14 days in an incubator in the dark at 28 °C. The micro-morphology and sporulation were observed by optics microscopy (Olympus, Japan).

2.3. Preparation of actinomyces (ACT) extract

The identified *actinomyces* species were cultivated on starch nitrate agar plate medium at 28 °C for 7–14 days (until complete sporulation). One-liter Erlenmeyer flasks, each containing 250 ml of ISP2 medium consisting of 4 g/l glucose, 4 g/l yeast extract, and 10 g/l malt extract, were inoculated with spore suspension from well grown slants (only one slant was used to inoculate two flasks). The flasks were incubated at 30 °C using rotary shaker (150 rpm) for 15 days. The cells were separated by centrifugation at 5000 rpm and 4 °C and both the cell-free supernatant and the cells biomasses were subject to extraction. The supernatant was extracted with ethyl acetate 3 times. However, the cell biomasses were extracted with acetone then the acetone was evaporated under vacuum and the remaining water residue was re-extracted 3 times with acetyl acetate (Shaaban et al., 2013).

2.4. Identification of chemical composition of the crude extracts of using GC-MS

The crude extract was analyzed by a coupled Varian gas chromatography/mass spectrometry (Perkin Elmer Auto XL GC, Waltham, MA, USA) equipped with a flame ionization detector to identify their chemical composition. The GC conditions were EQUITY-5 column (60 m × 0.32 mm x 0.25 μm); H_2 carrier gas; column head pressure 10 psi, the oven temperature was maintained initially at 70 °C for 2 min, and then programmed from 70 to 250 °C at a rate of 3 °C/min. The ionization voltage was 70 eV and mass range m/z 39–400 amu. The identification of individual compounds was based on their retention times relative to those of authentic samples and matching spectral peaks available with the published data (Iwasa et al., 2015).

2.5. Experimental animals

Two months old Sprague-Dawley female rats (100–110 g) were purchased from the Animal House Colony, Giza, Egypt and were maintained on standard lab diet (protein: 160.4; fat: 36.3; fiber: 41 g/kg and metabolizable energy 12.08 MJ, purchased from Meladco Feed Co., Aubor City, Cairo, Egypt) in artificial illuminated and temperature controlled room free from any other source of chemical contamination in the Animal House Laboratory, National Research Center, Dokki, Cairo, Egypt. After an acclimatization period of 1 week, the animals were divided into six groups (10 rats/group) and housed in filter-top polycarbonate cages. All animals 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).