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Quercetin inhibits the cytotoxicity and oxidative stress in liver of rats fed aflatoxin-contaminated diet

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ABSTRACT

This study was conducted to evaluate the protective role of quercetin (Q) against the cytotoxicity, DNA damage and oxidative stress in rats fed aflatoxin (AFs)-contaminated diet. Female Sprague–Dawley rats were divided into six groups and treated for 21 days as follows: the control group; the group fed AFs-contaminated diet (1.4 mg/kg diet); the groups treated orally with Q at low or high dose (50 and 100 mg/kg b.w.) and the groups AFs-contaminated diet plus low or high dose of Q. At the end of experiment, blood and liver samples were collected for biochemical, histological, histochemical and genetic analyses. The results indicated that animal fed AFs-contaminated diet showed significant increase in serum biochemical parameters, oxidative stress markers and DNA fragmentation accompanied with significant decrease in total proteins, GPX, SOD, DNA and RNA content and fatty acid synthase (Fas) and TNFα gene expression in the liver tissue. Q at the two tested doses succeeded to normalize the biochemical parameters, improved the content of nucleic acids in hepatic tissues, the gene expression, the histopathological and histochemical picture of the liver. It could be concluded that Q has a potential antioxidant activity, a protective action and regulated the alteration of genes expression induced by AFs.

1. Introduction

Aflatoxins (AFs) are major class of mycotoxins produced mainly by two species of *Aspergillus*, *Aspergillus flavus* and *Aspergillus parasiticus*. AFs have been associated with several toxic effects in animal and human health including carcinogenic, mutagenic, teratogenic and immunosuppressive activity [2,6,23]. AFs are one of the most potent toxic substances that are found in a wide range of agricultural crops especially grains and nuts which are commonly used for the preparation of different foods [14]. Although 20 aflatoxins have been identified, only 4 of them, i.e. the aflatoxins B_1, B_2, G_1 and G_2 (AFB_1, AFB_2, AFG_1 and AFG_2), occur naturally and are significant contaminants of a wide variety of foods and feeds. AFB_1 is the most potent carcinogenic substance and has continued to receive major research attention as the most carcinogenic and toxic mycotoxins [16,30]. It is classified by the International Agency of Research on Cancer as Group 1 human carcinogen [30]. The toxic metabolites AFB_1-8,9-epoxide resulted from biotransformation of AFB_1 by liver microsomal enzymes and the toxic effects of aflatoxins mostly arise from the binding of this particular epoxide derivative to DNA [48].

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It has been well documented that drug-metabolizing enzymes (phase-I and phase-II enzymes) and AFB_1-adduct formation can be changed by natural constituents of the diet, nutrients, phytochemicals and xenobiotics [1,3]. Recently, a great deal of attention has focused on the protective biochemical functions of naturally occurring antioxidants in biological systems against oxidative stress in liver tissue. Scavenging of free radicals by antioxidants could reduce the fibrosis process in the tissues also free radicals may act as a contributory factor in a progressive decline in the function of immune system [24]. Cooperative defense systems that protect the body from free radical damage include the antioxidant nutrients and enzymes [3,53].

A highly promising candidate for the prevention of adverse health effects in humans is quercetin (3,3′,4′,5,7-pentahydroxyflavone), one of the most abundant flavonoids in the human diet, which is found in fruits and vegetables such as blueberries, onions, curly kale, broccoli, and leek [37]. It is well documented that quercetin has broad bioactivity, such as antioxidative, hypolipidemic properties [9,10], ROS scavenging, anti-inflammatory and anti-fibrotic properties [29,36]. Moreover quercetin exhibits hepatoprotective effect against ethanol hepatotoxicity by counteracting oxidative stress *in vivo* [18,39,59] and *in vitro* [35,63]. The aims of the current study were to evaluate the protective role of aqueous extract of quercetin against oxidative stress, the cytotoxicity and DNA damage in liver tissue of rats fed AFs-contaminated diet.

2. Materials and methods

2.1. Chemicals and kits

Aflatoxins (AFs) standards and quercetin 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). Dimethyl sulfoxide (DMSO) was supplied by Merck (Germany). Triglycerides, total proteins, glutathione peroxidase (GPx), superoxide dismutase (SOD), alkaline phosphatase (ALP), nitric oxide (NO), lactate dehydrogenase (LDH), carcinoembrionic antigen (CEA), total lipid, cholesterol and lipid peroxide formation as malondialdehyde (MDA) were determined using kits purchased from Biodiagnostic Co. (Giza, Egypt). All other chemicals used throughout the experiments were of the highest analytical grade available.

2.2. Aflatoxins production

The aflatoxins (AFs) were produced through the fermentation of maize by *Aspergillus parasiticus* NRRL 2999 as described by [54]. The fermented maize was autoclaved; ground to a fine meal, and the AFs content was measured by the use of HPLC [28]. The AFs within the maize meal consisted of 45% B_1, 12% B_2, 30% G_1, and 13% G_2 based on total AFs in the maize powder. The maize meal was incorporated into the basal diet to provide the desired level of 1.4 mg of total AFs/kg diet. The diet containing AFs was analyzed and the presence of parent AFs was confirmed by HPLC. The safety measures recommended by [61] were taken when handling the AFs-contaminated diet.

2.3. Experimental animals

Three-month-old female Sprague–Dawley rats (100–150 g each) were purchased from Animal House Colony, National Research Centre, Dokki, Giza, Egypt. Animals were maintained on the specified diet 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 ($25 \pm 1\,^{\circ}C$) at the Animal House Lab., National Research Centre. All animals were received humane care in compliance with the guidelines of the Animal Care and Use Committee of the National Research Centre, Dokki, Giza, Egypt.

2.4. Experimental design

Animals were divided into six groups (8 rats/group) and were maintained on their respective diet for 3 weeks as follow: Group 1, normal control fed on basal diet and received 0.5% DMSO at a dose of 1 ml/kg b.w. by gastric tube; Group 2, animals fed AFs-contaminated diet (1.4 mg/kg diet); Group 3, animals treated daily with low dose of quercetin (50 mg/kg b.w.) dissolved in 0.5% DMSO by gastric tube; Group 4, animals treated daily with high dose of quercetin (100 mg/kg b.w.) dissolved in 0.5% DMSO by gastric tube; Groups 5 and 6, animals fed AFs-contaminated and treated orally with low and high dose of quercetin, respectively. The animals were observed daily for signs of toxicity during the experimental period. 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 $-20\,^{\circ}C$ until analysis. The sera were used for the determination of ALT, AST, ALP, LDH, NO, CEA, total protein, cholesterol, triglycerides and total lipid according to the kits instructions using Jenway spectrophotometer 6715 (Staffordshire, UK).

After the collections of blood samples, animals were sacrificed by cervical dislocation and samples of the liver of each animal were dissected, weighed and homogenized in phosphate buffer (pH 7.4) to give 20% (w/v) homogenate according to [34]. This homogenate was centrifuged at 1700 rpm and $4\,^{\circ}C$ for 10 min; the supernatant was stored at $-70\,^{\circ}C$ until analysis. This supernatant (20%) was used for the determination of hepatic lipid peroxide formation as malondialdehyde (MDA) and it was further diluted with phosphate buffer solution to give 2% and 0.5% dilutions for the determination of hepatic glutathione peroxidase (2%) and superoxide dismutase (0.5%) activities.

Other samples of the liver from all animals were fixed in 10% neutral formalin and paraffin embedded. Sections (5 μm thickness) were stained with hematoxylin and eosin (H & E) for the histological examination. Other sections from liver were stained with Bromophenol blue for the determination of protein content in liver tissue [22]. Another sample of the liver of each animal within different treatment groups was fixed in 3% glutaraldehyde,