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Aflatoxin B_1 induced renal and cardiac damage in rats: Protective effect of lycopene

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ABSTRACT

This study was conducted to investigate the protective effects of lycopene against the toxic effects of Aflatoxin B_1 (AFB_1) exposure in kidney and heart of rat by evaluating antioxidant defense systems and lipid peroxidation (LPO). Forty-two healthy three-month-old male Wistar-Albino rats were used in this study. The animals were randomly divided into six experimental groups including 7 rats in each. These groups were arranged as follows: control group, lycopene (5 mg/kg/day, orally for 15 days) group, AFB_1 (0.5 mg/kg/day, orally for 7 days) group, AFB_1 (1.5 mg/kg/day, orally for 3 days) group, AFB_1 (0.5 mg/kg/day, orally for 7 days) + lycopene (5 mg/kg/day, orally for 15 days) group and AFB_1 (1.5 mg/kg/day, orally for 3 days) + lycopene (5 mg/kg/day, orally for 15 days) group. The animals were sacrificed at the end of applications. In this study, malondialdehyde (MDA) levels significantly increased; while reduced glutathione (GSH), glutathione-S-transferase (GST), catalase (CAT), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and glucose-6-phosphate-dehydrogenase (G6PD) activities decreased in kidney and heart tissues. The significant reduction in the activities of antioxidant enzymes and non-enzymatic antioxidant system in AF treated rats as compared to the control group could be responsible for increased MDA levels observed during AF induced kidney and heart damage. The results showed increased urea, creatinine levels, as well as reduction sodium concentrations in plasma of AFB_1 treated rats. There was lycopene showed protection against AF induced nephrotoxicity and cardiotoxicity.

1. Introduction

Aflatoxins (AFs) are mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* as secondary metabolites (Corcuera et al., 2011). Humans and animals can be subject of AFs both directly and indirectly. AF has been recognized as a significant food contaminant in human food and animal feed and AFs are found in milk, eggs and edible tissues, after ingestion of AF-contaminated food from farm animals (Giovannucci, 1999). Especially, AFB_1 is the most highly toxic. Aflatoxins are well known to be potent mutagens, hepatotoxic, hepatocarcinogenic, nephrotoxic, teratogenic, genotoxic and immunosuppressive and also inhibit several metabolic systems, causing liver, kidney and heart damage. These toxins have been incriminated as the cause of high mortality in livestock and some cases of death in human being (Abdel-Wahhab et al., 2002; Bintvihok et al., 2002; Golli-Bennour et al., 2010). Acute exposure to a high dose of AFB_1 produced acute toxicity which may cause acute hepatotoxicity (Abdin et al., 2010; Azziz-Baumgartner et al., 2005), whereas chronic exposure to low doses of AFs is one of the major risk factors in the etiology of hepatocellular carcinoma. Although the principal toxic target for AFB1 is the liver in humans and animals, several studies indicated that AFB1 also causes severe heart damage (Mohamed and Metvvally, 2009; Abdulmajeed, 2011; Mannaa et al., 2014). However, the precise mechanism for AFB1 induced cardiotoxicity is incompletely known. It is well known that cardiomyocytes are particularly susceptible to attack by free radicals because the activity of antioxidant defense mechanism is lower than in other tissues. The kidneys are also considered as indefensible targets. Therefore, many reports supported the role of AFB_1 as a possible cause in kidney diseases (Bintvihok et al., 2002; Rati et al., 1991, Yilmaz et al., 2017a, 2017b).

Oxidative stress consists in a cell or tissue when the concentration of reactive oxygen species (ROS) generated exceeds the antioxidant capability of that cell (Trush and Kensler, 1991). The studies show that the occurrence of liver, kidney and heart disorders and oxidative damage or oxidative stress is increasing due to changing lifestyles causing serious problem in the area of public health (Devendran and Balasubramanian, 2011). Oxidative damage in the tissue occurs when the concentration of ROS (superoxide radical ($O^{\cdot}_2$), hydrogen peroxide (H_2O_2), and hydroxyl

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radical (·OH)) generated exceeds the antioxidant capacity of the cell or when the antioxidant capacity of the cell decreases. Antioxidants have their ability to reduce oxidative damage by either induction or inhibition of key enzyme systems (Guarisco et al., 2007). AF causes oxidative stress by increasing lipid peroxidation (LPO). Levels of non-enzymatic antioxidants (reduced glutathione (GSH)) and enzymatic antioxidants (glutathione peroxidase (GSH-Px, glutathione-S-transferase (GST), glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT)) are the major determinants of the antioxidant defense mechanism of the cell (Ravinayagam et al., 2012). The concentration of the absorbed AFB1 was higher in gonads, liver, kidneys, spleen, thymus, endocrine gland, lung and brain (Marvan et al., 1983). Cytochrome P450 (CYP450) acts on AFB1 and produces AFB1–8, 9-epoxide (Smela et al., 2001) which is responsible for induction of oxidative stress of tissues (El-Bahr, 2015; Yilmaz et al., 2017a, 2017b), depletion of antioxidants (El-Bahr, 2015), formation of DNA adducts and tumor initiation (Choy, 1993).

The toxic effects of AFB_1 on blood biochemical parameters are exhibited through decreased concentrations of calcium, inorganic phosphate, sodium and potassium and an increase in urea, creatinine and uric acid (Gowda and Ledoux, 2008; Kubena et al., 1998).

Management of AF toxicity without any side effect is still a challenge to the medical field, as presently available drugs for aflatoxicosis have one or other adverse effects. In recent years, the herbal remedy for the unsolved medical problems is wining significance in the research field. Chemoprevention of toxicosis, oxidative damage using antioxidant is the subject of most studies. Lycopene, a kind of natural carotene that mainly exists in watermelon, tomatoes and other food, has a diversity of biological activity, such as aging prevention, cancer prevention, anti-inflammatories and oxidation. Lycopene has highly free radical scavenging capacity and efficient antioxidant (Agarwal and Rao, 1998; Giovannucci, 1999; Gowda and Ledoux, 2008; Stahl and Sies, 2005; Velmurugan et al., 2001).

The present study has been conducted in order to evaluate the protective effect of lycopene, its effect on some specific biochemical for renal function and oxidative parameters in AFB_1 induced nephrotoxicity and cardiotoxicity in rat.

2. Materials and methods

2.1. Chemicals and reagents

AFB_1 (CAS No:1162-65-8) was purchased from the CAYMAN Chemical Company (Michigan, USA). Lycopene 10% FS (Redivivo™) was produced by DSM Company (Heerlen, NETHERLANDS). GSH, GR, thiobarbituric acid (TBA), H_2O_2, nicotinamide adenine dinucleotide phosphate (NADPH) and other reagents were supplied from Sigma (St. Louis, MO, USA).

2.2. Preparation of AFB_1 and lycopene

AFB_1 was dissolved in dimethylsulfoxide (DMSO) /PBS (1/1, v/v) (pH:7.2), and then administered to the experimental animals. Lycopene 10% FS (Redivivo™) was dissolved in olive oil and administered at the dose of 5 mg/kg.

2.3. Experimental design

Forty-two healthy three-month-old male Wistar-Albino rats (250–300 g body weight) were used in this study. Animals were housed under standard laboratory conditions of light (12 h light-dark cycle); temperature, 25 ± 2 °C; fed with standard rat pellet diet and tap water ad libitum. The protocol for the use of animals was approved by the National Institutes of Health and Committee on Animal Research. AFB_1 in approximately 0.5 ml volume of vehicle (20% DMS) was orally given to the animals at the dose of 0.5 mg/kg bw/day, for 7 days and 1.5 mg/kg bw/day, for 3 days. Lycopene was suspended in olive oil and administered to the animals by gavage at the dose of 5 mg/kg bw/every other day, for 15 days. The doses of AF and lycopene studies (Cohen, 2002; Kumagai et al., 1998; Miyata et al., 2004; Reginald et al., 1997; Yilmaz et al., 2006; Yilmaz et al., 2017a, 2017b) used in this study were selected from previous studies. The animals were randomly divided into six experimental groups including 7 rats in each. These groups were arranged as follows: Group 1- Control (not treated), Group 2- Lycopene (5 mg/kg bw/day, orally for 15 days) treated rats, Group 3- AFB_1 (0.5 mg/kg bw/day, orally for 7 days) treated rats, Group 4- AFB_1 (1.5 mg/kg bw/day, orally for 3 days) treated rats, Group 5- AFB_1 (0.5 mg/kg bw/day, orally for 7 days) + lycopene (5 mg/kg/day, orally for 15 days) treated rats, Group 6- AFB_1 (1.5 mg/kg bw/day, orally for 3 days) + lycopene (5 mg/kg/day, orally for 15 days) treated rats. AFB_1 was administered to three-month-old rats by gavage at the doses of 0.5 mg/kg bw /day for 7 days and 1.5 mg/kg bw/day for 3 days.

2.4. Biochemical analyses

At the end of the experiment, all animals were sacrificed and tissue samples were removed and immediately the kidney and heart tissues samples were stored at −80 °C until analyses were performed. Before of the analysis, tissues were washed with cold distilled water to remove blood and then homogenized in distilled water using a Potter-elvehjem homogeniser. The homogenate was centrifuged (3.000*g*, for 15 min to MDA, GSH, GST, CAT, SOD analyzes and 10.000 g for 55 min to GSH-Px at +4 °C). Blood containing EDTA samples were centrifuged at 3000 *g* for 15 min at +4 °C to obtain plasma. Plasma was used for urea, creatinine sodium, chloride and phosphorus levels analyze.

The MDA and GSH levels, GST, CAT, GSH-Px, SOD and G6PD activities were analyzed in kidney and heart tissues. MDA levels were measured according to the method of Placer et al. (1966). The concentrations of reagent (10%, *w/v* trichloroacetic acid and 0.168%, w/v TBA) were mixed in a glass tube. The solution was warmed for 20 min. The precipitate was removed by centrifugation at 4000*g* for 10 min and then absorbance of the supernatant was read at 532 nm on a spectrophotometer. The MDA level was expressed as nmol/g tissue. GSH level was determined by a kinetic assay using a dithionitrobenzoic acid (DTNB) recycling method of Ellman et al. (1961). GSH level was expressed as μmol/ml. CAT activity was done by Aebi's method (Aebi, 1984). The tissue CAT activity was determined according to Aebi (1984). The principle of the assay is based on the determination of the rate constant, k (dimension: k) of H_2O_2 decomposition. The reaction contained 50 mM potassium phosphate buffer and 10 mM H_2O_2 (as substrate) reaction was started by the addition of the sample. GSH-Px activity was measured by Beutler method (Beutler, 1983) which records the disappearance of NADPH through its absorbance at 340 nm. The GSH-Px activity was expressed as IU/g protein. GST activity was measured by the method of Habig et al. (1974). GST activity was expressed as IU/mg protein. SOD activity was measured using xanthine and xanthine oxidases to generate superoxide radicals which react with nitroblue tetrazolium (Sun et al., 1988). One unit of SOD activity was defined as the amount of enzyme required to cause inhibition of nitroblue tetrazolium. SOD activity was expressed as IU/mg protein. G6PD activity was measured by Beutler method (Beutler, 1983). The G6PD activity was expressed as IU/g protein. The plasma levels of urea, creatinine sodium, chloride and phosphorus were measured in auto analyzer (Olympus AU 600, Tokyo, Japan).

2.5. Histopathological examination

At the end of the experiment, necropsy of the rats performed kidney and heart tissue samples were fixed in 10% neutral buffered formalin. Paraffin embedded blocks were routinely processed and 5 μm thick sections were stained with haematoxylin-eosin and examined under an microscope and randomly 10 microscopic fields were examined in x40