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ORIGINAL PAPER

Effects of *Tarantula cubensis* D6 on aflatoxin-induced injury in biochemical parameters in rats

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Introduction: **Aflatoxins are toxic fungal metabolites that have adverse effects on humans and animals. *Tarantula cubensis* D6 is used as a homeopathic medicine for different purposes. The present study investigates the effects of *Tarantula cubensis* D6 on the oxidant-antioxidant balance and some biochemical parameters against exposure to aflatoxin.**
Methods: **Thirty-two Sprague-Dawley female rats were used and evenly divided into four groups. Group 1 served as control. Groups 2, 3, and 4 received 200 μl/kg.bw/day *Tarantula cubensis* D6 (applied subcutaneously), 400 μg/kg.bw/day total aflatoxin (approximately 80% AF B_1, 10% AF B_2, 6 %AF G_1, and 4%AF G_2), and 200 μl/kg.bw/day *Tarantula cubensis* D6 plus 400 μg/kg.bw/day total aflatoxin, respectively, for 28 days. At the end of 28 days, blood samples and some organs (liver, kidney, brain, and spleen) were taken from all the animals. Oxidative stress markers (MDA, SOD, CAT, GSH-Px) and some biochemical parameters (glucose, triglyceride, cholesterol, BUN, creatinine, AST, ALT and ALP, total protein, albumin) were evaluated in blood samples and tissues.**
Results: **Aflatoxin caused negative changes in all oxidative stress parameters and some biochemical parameters (glucose, triglyceride, cholesterol, creatinine, AST, ALT, ALP, total protein, albumin). Administration of Tarantula cubensis D6 partly alleviated aflatoxin-induced negative changes.**
Conclusions: **Our results indicated that *Tarantula cubensis* D6 partially neutralized the deleterious effects of aflatoxin.** *Homeopathy* (2015) **104**, 205–210.

Key words: Aflatoxin; *Tarantula cubensis* D6; Oxidative stress; Biochemical parameters; Rat

Introduction

Aflatoxins (AF) are secondary metabolites, naturally occurring mycotoxins produced especially by *Aspergillus flavus* and *Aspergillus parasiticus*. They are also known as potential contaminants in human and animal food. The aflatoxin species (AFB_1, AFB_2, AFG_1, and AFG_2) are visible under ultraviolet light in different colors. Cases of contamination are based on different variables, such as humidity temperature, the amount of fungi in the environment, physical damage, and storage conditions. AFB_1 is metabolized to AFB_1-exo-8,9-epoxide by the cytochrome P-450 enzyme system. This extremely reactive metabolite binds to DNA guanines to form adducts, causing toxic effect.[1–4] The effects of aflatoxins on multiple organs, such as oxidative stress, and other toxicities have been previously described in various experiments on animals.[5–10]

The homeopathic medicine *Tarantula cubensis* D6 (syn.: *Mygale cubanensis*) is nominally prepared from the Cuban tarantula. The spider is a member of the genus *Mygale*, which consists of large, mouse-shaped hairy tarantulas.

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Venoms of spiders of the genus *Loxosceles* cause serious arachnidism, a dangerous systemic reaction including renal failure, severe intravascular coagulation, thrombocytopenia, coma, and convulsions.[11]

Theranekeron® is a homeopathic product which contains *Tarantula cubensis* D6. Some positive effects including such as demercative, regenerative, anti-inflammatory and resorptive effects are defined in many previous studies.[12–18] *Tarantula cubensis* D6 in homeopathic medicine has been used successfully in the treatment of oral lesions in cattle with bluetongue disease,[12] canine oral papillomatosis,[13] canine mammary tumors,[14] indolent ulcers in cats,[15] thrombus in the left anterior descending artery,[16] foot-and-mouth disease in cattle,[17] and endometriosis.[18]

The effects of administration of *Tarantula cubensis* D6 on aflatoxin-induced biochemical changes in rats were evaluated on the basis of some oxidative stress marker and other biochemical parameters. There are many studies related to aflatoxin intoxication in rats[9,19–22] and other animals.[5–7,10] Several treatments are used in detoxification of aflatoxin.[8,23–26] But their benefit are not satisfactory. Oxidative stress is one mechanism in the formation of aflatoxin intoxication.[27] The effects of *Tarantula cubensis* D6 on cellular oxidative stress and other biochemical parameters in the case of exposure of aflatoxin has not previously been studied. The present study aims at evaluating the effectiveness of *Tarantula cubensis* D6 against aflatoxin-induced toxications in rats.

Materials and methods

Chemicals

The chemicals used in the experiments and analysis were purchased from Sigma–Aldrich (St Louis, MO, USA) or Merck (Darmstadt, Germany). *Tarantula cubensis* D6 (Theranekron®) was obtained from Richter Pharma AG Wels, Austria.

Aflatoxin production

Aflatoxin was produced in rice by using the *Aspergillus parasiticus* (NRLL 2999) strain according to the method of Shotwell *et al.*[28] as modified by Demet *et al.*[29] The extraction/purification and type analyses of produced aflatoxins from rice flour were carried out according to the R-Biopharm Ridascreen® kit procedure for total aflatoxin and Nabney and Nesbit,[30] respectively. The percentages of AFB_1, AFB_2, AFG_1, and AFG_2 in extract were found to be approximately 80%, 10%, 6%, and 4%, respectively. The level of total aflatoxin was estimated by means of the R-Biopharm Ridascreen® total aflatoxin kit and the method suggested by the producer in extract. Following purification of aflatoxin, organic solvent containing aflatoxin was evaporated. Then aflatoxin was solved in dimethysulfoxide/water (4:6, v/v) for using experiment.

Animal material

Thirty-two 4- to 5-week-old (200–250 g) Sprague-Dawley female rats were used and divided evenly into four groups. The first group was control. Groups 2, 3, and 4 were given 200 μl/kg.bw/day *Tarantula cubensis* D6 (Theranekron®), 400 μg/kg.bw/day total aflatoxin, and 200 μl/kg.bw/day *Tarantula cubensis* D6 plus 400 μg/kg.bw/day total aflatoxin, respectively, for 28 days. Aflatoxin was administered early in the morning via oral gavage. *Tarantula cubensis* D6 was administered after 6 h the same day by subcutaneous route. Number of animals used in this study was based on earlier aflatoxin studies.[8] Determination of oral aflatoxin[31,32] and *Tarantula cubensis* D6 dose,[13] previous studies were based on but some modifications were made on application period for *Tarantula cubensis* D6 considering the severity of aflatoxin intoxication. The protocol of the present study was approved by the Ethics Board for Experimental Animals of Erciyes University.

Sample collection and preparations of analysis

Blood samples were withdrawn into tubes (without/with anticoagulant) from all animals under a light ether anesthesia. The collected blood samples were centrifuged at 3000 rpm for 10 min at +4°C (Sigma 3K30, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany), and their erythrocytes, plasma and sera were separated. The erythrocytes were washed in saline phosphate buffer (PBS), diluted with equal volumes of PBS and were lysed with ice-cold water.[33]

Following the collection of blood samples and their deaths, the liver, kidneys, brain and spleen were taken from all the animals. All extracted organs were washed in cold distilled water. Than fat and connective tissues were then removed. The tissues were homogenized with phosphate buffer adjusted to a pH value of 7.4, using a homogenizer (Heidolph, SilentCrusher M) and then the homogenized tissues were centrifuged at 15,000 rpm for 45 min at +4°C, and the supernatant was placed in eppendorf tubes. All samples were stored at −80°C until the analysis.

Assessment of oxidative stress parameters

Plasma/tissue malondialdehyde (MDA) levels,[34,35] blood hemoglobin levels,[36] tissue protein levels,[37,38] tissue superoxide dismutase (SOD) activities[39], catalase (CAT) activities,[40] glutathione peroxidase activities (GSH-Px)[41] were determined according to the mentioned methods. The MDA results were expressed in units of nmol/ml or nmol/mg-protein. Units of enzymes for tissues SOD, CAT, and GSH-Px were expressed as U/mg protein, U/g protein, and U/g protein, respectively. Units of enzymes for erythrocyte SOD, CAT, and GSH-Px were indicated as U/mgHb, U/gHb and U/gHb, respectively. All oxidative stress markers and tissue protein levels were measured spectrophotometrically (Helios α, double-beam UV/VIS).

Measurement of biochemical parameters in sera

Detection of serum glucose, triglyceride, cholesterol, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT),

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