Bull. Environ. Contam. Toxicol. (1998) 60:626-631
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Bulletin of Environmental Contamination and Toxicology

Hyperglycemia Induced in Rabbits Exposed to Ochratoxin

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Received: 19 November 1997/Accepted: 29 January 1998

Ochratoxins are secondary toxic fungal metabolites produced by *Aspergillus ochraceus* and *Penicillium viridicatum*. The problem of ochratoxin contamination in foodstuffs has received considerable attention in recent years (Tsubouchi et al. 1988; Hult et al. 1992; Breitholt et al. 1993; Zohri and Saber 1993; Ruprich and Ostry 1993). Ingestion of an ochratoxin-contaminated diet causes a toxic disease called ochratoxicosis. Ochratoxin is primarily a nephrotoxin (Krogh 1987), but hepatoma and hepatocarcinoma were also observed in albino rats (Rati et al. 1991). Our own earlier work indicates that feeding ochratoxin-contaminated diets (10 mg/kg) to rabbits for 90 days causes derangement of liver structure, slight to moderate congestion of the central vein, focal necrosis, and fatty infiltration of the liver (Verma et al. 1997).

The liver functions as a very important blood-glucose buffer system. In patients with severe liver disease, it becomes almost impossible to maintain blood glucose concentrations for proper functioning of the brain and retina (Guyton 1981). Although the liver is severely affected during ochratoxicosis, changes in blood glucose levels remain unknown. The present investigation evaluated changes in blood glucose level during ochratoxicosis in rabbits.

MATERIALS AND METHODS

Toxigenic strain of *Aspergillus ochraceus* (ITCCF 1456 obtained from Indian Agricultural Research Institute, New Delhi) was grown on yeast extract-sucrose liquid medium for 10 days at 28±2°C (El-Shayeb et al. 1992). Culture filtrates were pooled and extracted twice with analytical grade chloroform (1:2, v/v), passed through the bed of anhydrous sodium sulfate, evaporated to dryness, and analyzed for its toxin content. TLC plates uniformly coated with silica gel G and activated at 120°C for 1 hr were spotted with 100 µl extract alongwith a pure ochratoxin A (a gift from Dr. Karl Hult of Royal Institute of Technology, Stockholm, Sweden) as a standard. Chromatoplates

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were developed in a solvent system comprised of toluene : ethyl acetate : formic acid (50 : 40 : 10, v/v) (Vorster 1969) and observed under long-wave UV light (360 nm). Chemical confirmation of ochratoxins were also done by spreading a developed chromatoplate with potassium hydroxide or ammonia solution on the field (Vorster 1969). Each spot was subsequently scrapped separately and dissolved in 5 ml chilled spectrophotometric methanol and processed for quantitative analysis of ochratoxin using a Shimadzu UV-160A spectrophotometer (Vorster 1969).

The crude ochratoxin concentrate in chloroform was thoroughly mixed with feed (as per formulations of National Institute of Occupational Health, Ahmedabad, India) to get a concentration of 10 mg/kg of feed. Feed was left overnight for complete evaporation of chloroform. Ration for control rabbits was similarly treated with chloroform alone and left overnight for evaporation of chloroform and was analyzed for absence of toxins.

Young inbred New Zealand strain of female rabbits (*Oryctolagus cuniculus* obtained from Cadila Research Laboratories, Ahmedabad, India) weighing approximately 700-750 g were provided with feed and water *ad libitum.* Ten such female rabbits were segregated in two groups: while Group 1 rabbits were fed with ochratoxin-contaminated feed (10 mg/kg) continuously for 90 days; control animals received non-toxic feed.

On completion of the treatment, rabbits were sacrificed by cervical dislocation. Blood samples collected from jugular vein were analyzed for glucose and lactate concentrations by standard methods of Folin and Malmros (1929) and Baker and Summerson (1941), respectively. Urine samples collected from the urinary bladder were analyzed for glucose and lactate content by the methods described above.

Liver was quickly excised, blotted free of blood, and weighed. Small pieces of liver of known weight were digested in 30% KOH and processed for the estimation of glycogen employing anthrone method as described by Seifter et al. (1950). Lipid was extracted from powdered dry tissues employing the method of Folch et al. (1957) using a mixture of chloroform and methanol (2 : 1, v/v). The extracted lipid was measured gravimetrically and expressed as gm % non-fat dry tissue (NFDT). Lactate dehydrogenase (E.C. 1.1.1.27) activity was assayed by the method of King as described by Varley (1988) using sodium lactate as substrate. Activities of aspartate aminotransferase (AST) (E.C.2.6.1.l) and alanine aminotransferase (ALT) (E.C.2.6.1.2) were assayed by the method of Bergmeyer and Bernet (1965). Protein content of all homogenates was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Student's 't' test was used for statistical analysis of the data.

RESULTS AND DISCUSSION

Consumption of ochratoxin-contaminated diets by rabbits for 90 days caused a significant rise in blood glucose and lactate concentrations (Table 1).

Absolute and relative weights of the liver did not differ between ochratoxin-fed and control rabbits. Glycogen content was reduced and lipid content was increased during ochratoxicosis (Table 2).

Activity of lactate dehydrogenase, which converts lactate to pyruvate, increased significantly during ochratoxicosis (Table 3). This suggests an increased capacity of the liver to utilize lactate produced intracellularly or obtained through blood. While the activity of aspartate aminotransferase rose significantly, alanine aminotransferase was not influenced by ochratoxin ingestion (Table 3).

Significant increases in glucose and lactate were observed in the urine of ochratoxin-fed rabbits (Table 4).

The present results clearly indicated that consumption of ochratoxin-contaminated feed for 90 days caused hyperglycemia in rabbits. Hyperglycemia could occur by accelerating breakdown of glycogen in the liver (glycogenolysis), conversion of other nutrients into glucose in the liver (gluconeogenesis), and releasing glucose into blood. Ochratoxin has been found to reduce glycogen content not only in the liver but also in the skeletal muscles (Shalini 1996), which could be due to enhanced rate of glycogenolysis. In addition, activities of certain enzymes responsible for transamination of amino acids such as AST and ALT were increased in the liver. It indicates stepped up utilization of proteins/amino acid in the process of gluconeogenesis during ochratoxicosis. Lactate dehydrogenase is a cytoplasmic enzyme which helps regenerate glucose from lactate. Significant increase in LDH activity showed increased conversion of lactate to glucose.

Rabbits during ochratoxicosis became lethargic which could be explained by increased anaerobic metabolism. Reduced RBC count, haemoglobin content and packed cell volume (PCV) have been reported during ochratoxicosis in rabbits (Shalini 1996) indicating insufficient supply of oxygen to tissues. Ochratoxin-induced cytotoxicity to RBC has also been reported (Zofair et al. 1996). Increased blood lactate during ochratoxicosis could be due to an enhanced rate of anaerobic metabolism.

Table 1. Effects of feeding ochratoxin-contaminated diets for 90 days on concentrations of blood glucose and lactate in rabbits

Parameters	Control	Treated
Glucose (mg/dl)	112.2 ± 2.2	143.0 ± 3.7*
Lactate (mg/dl)	20.6 ± 2.3	45.0 ± 2.6*

N = 5; Values are mean ± S.E.M.;
Values are significant at * P < 0.001

Table 2. Liver mass and composition of rabbits fed ochratoxin contaminated diets for 90 days

Parameters	Control	Treated
Absolute liver weight (g)	26.6 ± 2.5	29.6 ± 2.6
Relative liver weight (g/100 g body weight)	2.5 ± 0.6	2.8 +±0.45
Glycogen (g % wet liver)	12.0 ± 0.2	3.8 ± 0.19*
Lipid (g % non-fat dry tissue)	36.8 ± 1.7	42.0 ± 0.6*

N = 5; Values are mean ± S.E.M.;
Values are significant at * P < 0.001

Induced ochratoxicosis in rabbits caused rise in blood glucose and lactate followed with their increased appearance in urine. We conclude that consumption of ochratoxin-contaminated feed has hyperglycemic effects in rabbits.

Acknowledgments: Financial assistance received from Gujarat Government, Gandhinagar, India is thankfully acknowledged. The authors are also grateful to Dr. Karl Hult, Royal Institute of Technology, Stockholm, Sweden for providing samples of pure ochratoxin A.

Table 3. Effects of feeding ochratoxin-contaminated diets for 90 days on certain liver enzymes in rabbits

Parameters	Control	Treated
Lactate dehydrogenase[1]	4.25 ± 0.24	5.40 ± 0.17*
Alanine aminotransferase[2]	0.52 ± 0.04	0.55 ± 0.02
Aspartate aminotransferase[2]	0.44 ± 0.04	0.44 ± 0.03**

N = 5; Values are mean ± S.E.M.;
Values are significant at * P < 0.01; ** P < 0.001
1. µ mole lactate oxidized/mg protein/30 min
2. Karmen units/mg protein/min

Table 4. Effects of feeding orchatoxin-contaminated diets for 90 days on concentrations of glucose and lactate in urine

Parameters	Control	Treated
Glucose (mg/dl)	80.0 ± 6.3	192.2 ± 7.2*
Lactate (mg/dl)	6.2 ± 4.3	26.3 ± 2.3*

N = 5; Values are mean ± S.E.M.;
Values are significant at * P < 0.001

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