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Ochratoxin A Toxicity on Carbohydrate Metabolism in Rats

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Ochratoxin A is a toxic secondary metabolite produced by several species of Aspergillus and Penicillium (Ciegler et al. 1972). The distribution of these fungi in the natural environment is extensive and are reported to contaminate a wide variety of foods and feeds (Prior 1976). Ochratoxin A has been reported to be hepatotoxic, teratogenic and nephrotoxic (Prior et al. 1983). Previously we have reported that ochratoxin A ingestion is found to increase blood glucose in chicks (Subramanian and Govindasamy 1985). Hence the present work was undertaken to understand the effect of ochratoxin A on glucose metabolism in rats.

MATERIALS AND METHODS

The strain Aspergillus ochraceus was isolated in our laboratory from a fungal contaminated feed and it was confirmed by Indian Agricultural Research Institute, New Delhi, India. The strain was found to produce ochratoxin A as a major secondary metabolite. Ochratoxin A was isolated from the culture filtrate of A. ochraceus using the method of Suzuki et al. (1975). An authentic sample of ochratoxin A supplied by Dr. Timothy D. Phillips, Department of Veterinary Public Health, Texas A and M University, U.S.A. was used as reference.

Thirty weanling albino rats from our laboratory animal colony weighing 120 ± 15 g were used in the investigations. The animals were maintained on standard diet (Hindustan Lever Ltd., Bombay, India) and water 'ad lib'. The rats were divided into two groups; each comprising of 15 rats. The first group of rats were administered orally with 0.5 M $NaHCO_3$ solution containing 100 µg of ochratoxin A per rat daily and the second group of rats received the same

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amount of 0.1 M $NaHCO_3$ solution (vehicle) everyday for eight wks.

At the end of the experimental period, the animals were sacrificed and the blood was collected from the aorta in centrifuge tubes to separate serum. Blood glucose concentration was determined according to the method of Sasaki et al. (1972). Serum insulin level was assayed according to the method described by Herbert et al. (1965). The liver tissue was dissected out and homogenised in 0.1 M Tris-HCl buffer (pH 7.4) and the homogenate was used for the assay of hexokinase (Brandstrup et al. 1957), aldolase (King 1965), glucose-6-phosphate dehydrogenase (Ells and Kirkman 1961), lactate dehydrogenase (King 1965), glucose-6-phosphatase (King 1965), fructose-1, 6-diphosphatase (Gancedo and Gancedo 1971), glycogen synthetase (Leloir and Goldemberg 1962), glycogen phosphorylase (Cornblath et al. 1963) and protein (Lowry et al. 1951). Another portion of the liver was used for the estimation of glycogen (Morales et al. 1973), pyruvate (Friedemann and Haugen 1943) and lactate (Barker and Summerson 1941).

RESULTS AND DISCUSSION

The values of blood glucose, serum insulin, lactate, pyruvate and glycogen levels in the liver of control and ochratoxin A administered rats are presented in Table 1.

Table 1: Blood glucose, serum insulin, lactate, pyruvate and glycogen levels in the liver of control and ochratoxin A administered rats.

	Control	Toxin administered
Blood glucose mg/100 ml	59.20 ± 4.80	112.40 ± 5.10***
Serum insulin IU/ml	21.30 ± 1.30	15.40 ± 1.80***
Liver lactate mg/g of wet tissue	15.64 ± 1.78	7.94 ± 2.20***
Liver pyruvate mg/g of wet tissue	0.46 ± 0.10	0.31 ± 0.07*
Liver glycogen mg/g of wet tissue	42.15 ± 3.15	20.50 ± 3.90***

Values are mean of six observations, ± indicates standard deviation of mean. A significant difference was observed as evaluated using the Student's t-test, where *$p < 0.02$, and ***$p < 0.001$.

From the observations, it is evident that ochratoxin A administered rats showed increased blood glucose level accompanied with lowered serum insulin level. The liver glycogen content was found to be depleted drastically. A marginal decrease in the levels of lactate and pyruvate were also noticed.

The increase in the blood glucose level may be due to decreased levels of insulin during ochratoxin A toxicosis which inturn may be due to inhibited synthesis and/or reduced release of insulin from the pancreatic β cells. Induction of hyperglycemia associated with hypoinsulinemia by mycotoxins like terreic acid (Shanmugasundaram et al. 1984) and penitrem A (Suseela et al. 1986) have been reported. Elevated blood glucose levels accompanied with depletion of hepatic glycogen in chicks during ochratoxin A toxicosis has also been noticed (Subramanian and Govindasamy, 1986). The lowered levels of lactate and pyruvate in the liver of experimental rats suggests the possible inhibition of glycolysis.

Liver functions as a 'glucostat' and plays a vital role in the maintenance of blood glucose level either by up-take or by release of glucose in the blood. Ochratoxin A has been reported to cause injury to liver (Subramanian et al. 1987). Hence it is of interest to examine the possible effect of ochratoxin A on the key enzymes of carbohydrate metabolism in liver. The activities of hexokinase, aldolase, lactate dehydrogenase, glucose-6-phosphate dehydrogenase, glycogen synthetase, glycogen phosphorylase, glucose-6-phosphatase and fructose-1,6-diphosphatase are presented in Table 2.

The activities of glycolytic enzymes such as hexokinase, aldolase and lactate dehydrogenase were found to be decreased whereas gluconeogenic enzymes such as glucose-6-phosphatase and fructose-1,6-diphosphatase were elevated during ochratoxin A toxicosis. A decrease in the activity of glycogen synthetase and an increase in the activity of glycogen phosphoryylase were also noticed.

The reduced level of glycogen content suggests the possible mobilization of carbohydrate reserve which is further supported by the enhanced activity of glycogen phosphorylase and reduced activity of glycogen synthetase. Reduction in the utilization of carbohydrates during ochratoxin A toxicosis is also evidenced by the observations of reduced activities of glucose-6-phosphate dehydrogenase and enhanced activities of glucose-6-phosphatase and fructose-1,6-diphosphatase.

Table 2 : Hexokinase, aldolase, lactate dehydrogenase, glucose-6-phosphate dehydrogenase, glycogen synthetase, glycogen phosphorylase, glucose-6-phosphatase and fructose-1,6-diphosphatase activities in the liver tissue of control and ochratoxin A administered rats.

	Control	Toxin administered
Hexokinase (m μ moles of G6P formed/hr/mg protein)	182 ± 11	136 ± 07***
Aldolase (μ moles of glyceraldehyde formed/ hr/mg protein)	578 ± 14	462 ± 16***
Lactate dehydrogenase (μ moles of pyruvate formed/hr/mg protein)	387 ± 17	272 ± 23***
Glucose-6-phosphate dehydrogenase (Change in absorbance/min/mg protein)	348 ± 16	261 ± 12***
Glycogen synthetase (n moles of UDP formed/ hr/mg protein)	652 ± 40	408 ± 28***
Glycogen phosphorylase (n moles of Pi liberated hr/mg protein)	182 ± 14	292 ± 16***
Glucose-6-phosphatase (μ moles of Pi liberated hr/mg protein)	1423 ± 46	2139 ± 67***
Fructose 1,6-diphosphatase (μ moles of Pi liberated/hr/mg protein)	152 ± 11	246 ± 18***

Values are mean of six observations, ± indicates standard deviation of mean. A significant difference was observed as evaluated using the Student's t-test, where ***$p < 0.001$.

The alterations observed in the present study on the activities of carbohydrate metabolising enzymes and the levels of carbohydrate metabolites may be attributed to the effective influence of ochratoxin A on insulin concentration. From the above observations we conclude that ochratoxin A is diabetogenic in nature and exerts its toxic effect by reducing the level of insulin and thereby suppressing glycolysis, glycogenesis and enhancing gluconeogenesis and glycogenolysis.

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