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Alterations in Bilirubin Concentrations during Induced Aflatoxicosis in Rabbits

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Aflatoxin is a well known mycotoxin produced by toxigenic strains of Aspergillus flavus - parasiticus group of fungi. Natural contamination of food/feed materials with aflatoxin have been recorded from almost every country, but in general higher concentrations were recorded from tropical and sub-tropical countries where environmental conditions are more congenial for mould growth and aflatoxin production.

Feeding aflatoxin contaminated diet (15 mg/kg feed) to rabbits caused significant reduction in red blood cell (RBC) count, hemoglobin concentration and packed cell volume (PCV) (Verma and Raval, 1992). Decreased RBC count in vivo may be due to the cytotoxic effect of aflatoxin (Verma and Raval, 1991) and may elevate the formation of bilirubin. Bilirubin is a greenish yellow toxic pigment formed during degradation of hemoglobin at the reticuloendothelial tissue and is excreted by the liver in the bile. Therefore, the present investigation was undertaken to examine bile content in the gall bladder and bilirubin concentrations in serum and urine during aflatoxin-induced toxaemia in rabbits.

MATERIALS AND METHODS

Toxigenic strain of A. parasiticus (NRRL 3240) was grown on SMKY liquid medium at 28±2°C for 10 days (Diener and Davis, 1966). Obtained culture filtrates were extracted with chloroform and the aflatoxin content was quantified using Shimadzu U.V.-160A spectrophotometer (Nabney and Nesbitt, 1965). The aflatoxin concentrate in chloroform was thoroughly mixed with the balanced diet to get a concentration of 15 mg aflatoxin/kg of feed. This toxin-mixed ration was left overnight to allow for chloroform evaporation. The presence of toxin in such toxin-mixed ration was ensured by taking random samples for analysis. Feed for control rabbits was similarly mixed with chloroform above and was analyzed to rule out any trace of aflatoxin (Verma and Raval, 1992).

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Table 1. Bile content in gall bladder during aflatoxicosis in rabbits

Experimental conditions	Liver weight (gm)		Bile content (gm)	
	With bile content	Without bile content	Absolute	Relative
Control	25.9650 ±2.6970	23.8926 ±2.5464	1.7391 ±0.1100	0.2810 ±0.0160
Treated	27.8038 ±2.7714	26.0974 ±2.6686	1.4815 ±0.1374	0.2470 ±0.0170

N=10; Values are Mean ± S.E.M.

Young inbred New Zealand strain of rabbits (Oryctolagus cuniculus weighing 200-225 gm (approximately 1 month old) were fed with ration and water ad-libitum, and maintained under laboratory conditions. For present experiment 20 rabbits were segregated into two groups. Group I rabbits were fed with aflatoxin-contaminated feed (15 mg/kg feed) for 60 days, while Group II animals received non-toxic feed and served as controls.

On completion of treatment, blood samples were collected from jugular vein and centrifuged to get serum. Urine samples were collected from urinary bladder with the help of a glass syringe. Serum and urine samples were analyzed for conjugated, unconjugated and total bilirubin using the Diazo method (Mukherjee, 1988). Bile content in the gall bladder was measured by weighing the intact liver with and without bile.

The Student's 't' test was used for statistical analysis.

RESULTS AND DISCUSSION

Bile content in the gall bladder of control rabbits was 1.731 gm which was reduced to 1.481 gm after feeding aflatoxin contaminated diet for 60 days. Relative bile content also showed a sharp decline during aflatoxicosis (Table 1).

Table 2 shows concentrations of conjugated, unconjugated and total bilirubin in serum and urine of rabbits during aflatoxicosis. While unconjugated bilirubin was more prevalent than conjugated ones in the serum, the reverse was true for urine in the control rabbits. Feeding aflatoxin-contaminated diet to rabbits caused a significant increase in total bilirubin content in the serum. Although significantly higher concentrations were noted in conjugated and unconjugated bilirubins in treated one, the increase in conjugated bilirubin was comparatively higher. In the urine of treated rabbits total bilirubin content showed a 2.5-fold increase ($P < 0.01$). Increases in conjugated and unconjugated bilirubins were 2.06 mg/dl and 3.1 mg/dl fold, respectively.

Table 2. Conjugated, unconjugated and total bilirubin concentrations in the serum and urine of aflatoxin-fed rabbits

Parameters	Control	Treated
Conjugated bilirubin in serum (mg/dl)	0.2662 ±0.0396	0.5806[b] ±0.1112
Unconjugated bilirubin in serum (mg/dl)	0.6028 ±0.0562	0.9174[c] ±0.1404
Total bilirubin in serum (mg/dl)	0.8690 ±0.0726	1.4980[a] ±0.1744
Conjugated bilirubin in urine (mg/dl)	2.3852 ±0.3516	4.9196[b] ±0.9018
Unconjugated bilirubin in urine (mg/dl)	1.7576 ±0.6054	5.5476[c] ±1.4356
Total bilirubin in urine (mg/dl)	4.1428 ±0.8588	10.4672[a] ±1.9700

N=10; Values are Mean ± S.E.M.
Values for the same parameter in the same row with different superscript's significantly differ at these levels:
[a]$P<0.01$; [b]$P<0.02$; [c]$P<0.05$.

The decrease in bile content in the gall bladder could be due to bile duct proliferation (Clark et al., 1980, 1982; Jaskiewicz et al., 1988; Harvey et al., 1989; Verma et al., 1991) and/or decreased transport from sinusoids to the gall bladder as a result of increased hepatocellular necrosis during aflatoxicosis.

The present results clearly indicate the occurrence of hyperbilirubinaemia during induced aflatoxicosis in rabbits. Some earlier reports also indicate a rise in the concentration of total bilirubin in the serum (Allcroft and Lewis, 1963; Sisk et al., 1968; Edds, 1973; Gumbmann and Williams, 1969; Osuna et al.,1977; Mertens, 1979 and Clark et al., 1980, 1982). Furthermore, the present data also indicate that the increase in total bilirubin concentration is due to increases in conjugated and unconjugated bilirubin. Aflatoxin treatment of an RBC suspension *in vitro* caused concentration-dependent swelling and hemolysis (Verma and Raval, 1991). Hepatocellular injuries are well known to increase concentrations of both conjugated as well as unconjugated bilirubin in the serum (Robbins and Cotran, 1979). Thus, hepatocellular changes, along with enhanced rates of hemolysis may be responsible for the increased conjugated and unconjugated bilirubins.

The present result also reveals increased levels of conjugated, unconjugated and total bilirubin in urine during aflatoxin-induced

toxaemia in rabbits. Occurrence of hyperbilirubinaemia and decreased excretion of bile by the liver, evident by decreased bile content of the gall bladder, may lead to their increased appearance in the urine.

It is concluded from the above results that induced chronic aflatoxicosis in rabbits caused hyperbilirubinaemia and appearance of bilirubin in the urine.

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