

## **Alterations in Erythrocytes during Induced Chronic Aflatoxicosis in Rabbits**

R. J. Verma and P. J. Raval

Department of Life Sciences, Bhavnagar University, Bhavnagar-364, 002, India

Aflatoxin is a secondary toxic fungal metabolite produced by toxigenic strains of Aspergillus flavus - parasiticus group of fungi. Occurrence of aflatoxin in various food commodities have been widely reported from various countries, being most prevalent in tropical and subtropical countries where environmental conditions are more favourable for mouldy growth and toxin production (Shank et al., 1972; Stoloff, 1977; Busby and Wogan, 1984). In parts of India 100% of maize samples have been found contaminated with aflatoxin content in the range of 6,250-15,600 µg/kg (Krishnamachari et al., 1975).

Ingestion of aflatoxin-contaminated feed cause serious toxic disease called aflatoxicosis in which symptoms like respiratory troubles and lethargy are well documented. Erythrocytes containing hemoglobin are highly specialized cells which serve as carrier of gases between lungs and tissues. Many investigators have examined the effect of aflatoxin on erythrocytes and its related parameters with conflicting results (Cysewski et al., 1968; Clark et al., 1980; 1984; Patterson, 1983; Bortell et al., 1983; Wyatt et al., 1985; Ray et al., 1986; Ranjan, 1987; Reddy et al., 1987; Harvey et al., 1988; Reddy and Sharma, 1989; Verma et al., 1991). The present experiment is an attempt to investigate alterations in erythrocytes and consequent effects during induced chronic aflatoxicosis.

### **MATERIALS AND METHODS**

Toxigenic strain of A. parasiticus (NRRL 3240) was grown on SMKY liquid medium for 10 days at  $28 \pm 2^\circ\text{C}$  as described by Diener and Davis (1966). Obtained culture filtrates were extracted with chloroform and concentration of aflatoxin was quantified (Nabney and Nesbitt, 1965).

Inbred New Zealand strain of rabbits (Oryctolagus cuniculus) weighing 200-225 gm were fed with ration and water ad-libitum and maintained under laboratory conditions. For present experiment 20 rabbits were divided in two groups; Group I rabbits

---

Send reprint requests to Dr.R.J.Verma at the above address

were fed with aflatoxin-contaminated diet (15 mg/kg feed) for 60 days, whereas Group II rabbits received non-toxic feed and served as control. Blood samples for hematological studies were collected in EDTA bulbs from ear-pinna of rabbits on 15, 30, 45 and 60 days of treatment. Erythrocytes were counted by hemocytometric method using Neubauer chamber. Packed cell volume (PCV) was measured by Wintrobe tube method. Concentration of hemoglobin was determined by acid hematin method. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated using standard formula (Mukherjee, 1988). Student 't' test was used for statistical analysis.

## RESULTS AND DISCUSSION

Data obtained in the present experiment is represented in Table 1. RBC count and hemoglobin concentration showed a decrease during aflatoxicosis. A continuous time dependent decrease could be observed upto 45 days of treatment. Significant decrease was recorded on 30, 45 and 60 days of treatment. PCV registered a time dependent continuous decrease. Data on 45 and 60 days of treatment were found to be significantly different from its control. A time dependent decrease in these parameters suggest occurrence of cumulative toxicity during aflatoxicosis. MCV showed a decline from its control values except on 45 days where a slight increase was noticed. Initial decline in MCH and MCHC (15 and 30 days) were followed with an increase on 45 and 60 days. Values were not found statistically significant from its control.

RBC count, hemoglobin concentration and PCV were significantly decreased during induced chronic aflatoxicosis (Cysewski et al., 1968; Panda et al., 1975; Clark et al., 1980; Patterson, 1983; Brucato et al., 1986; Ranjan, 1987; Reddy et al., 1987; Verma et al., 1991). However, in pigs (Harvey et al., 1988) cattle (Wyatt et al., 1985; Ray et al., 1986), goat (Clark et al., 1984) and pony (Bortell et al., 1983) increased levels of RBC, hemoglobin and PCV were reported. In BALB/c mice no effect on RBC counts were noted in AFB<sub>1</sub> exposure (Reddy and Sharma, 1989). These differences are believed to be due to species sensitivity, variations in dose and duration of the treatment (Huff et al., 1986; 1988). The present data also indicates time dependent decrease suggesting occurrence of cumulative toxicity during aflatoxicosis. Lower concentrations of aflatoxin may cause increase in RBC count, hemoglobin and PCV, whereas, higher concentration showed a decrease.

Exact mechanism causing decreased RBC count and related hemoglobin and PCV are not clearly understood. It could result from inhibition of hematopoiesis, defective hematopoiesis, increased rate of destruction of RBC or a combination of all three. Our in vitro studies have shown enhanced rate of aflatoxin induced morphological alterations and hemolysis when saline suspension of RBC was treated with aflatoxin (Verma and Raval, 1991).

Table 1. Erythrocytes and its related parameters of rabbits fed with 15 mg/kg aflatoxin-contaminated feed for 60 days

Day of treatment	RBC (x10 <sup>6</sup> cells/ $\mu$ l)	Parameters*				
		Hb (g/dl)	PCV (%)	MCV ( $\mu$ m <sup>3</sup> )	MCH (pg)	MCHC (%)
Control	5.86 ±0.45	11.63 ±0.30	40.68 ±1.03	75.41 ±3.43	21.48 ±0.95	28.53 ±0.50
15	5.08 ±0.28	11.27 ±0.25	39.55 ±1.07	74.51 ±5.91	20.78 ±1.41	28.28 ±0.97
30	4.79 <sup>a</sup> ±0.23	10.58 <sup>c</sup> ±0.20	38.90 ±0.77	70.51 ±4.18	20.85 ±1.59	27.28 ±0.75
45	4.58 <sup>b</sup> ±0.24	10.56 <sup>c</sup> ±0.13	37.14 ±0.84	77.97 ±4.04	23.05 ±1.53	29.54 ±0.49
60	4.66 <sup>a</sup> ±0.32	10.86 <sup>a</sup> ±0.19	36.44 <sup>c</sup> ±0.59	73.68 ±2.50	24.36 ±1.51	29.72 ±0.89

\* RBC=erythrocytes, Hb=hemoglobin, PCV=packed cell volume, MCV=mean corpuscular volume, MCH=mean corpuscular hemoglobin, MCHC=mean corpuscular hemoglobin concentration. N=10; Values expressed as mean  $\pm$  SE.

Values for the same parameter in the same row with different superscripts significantly differ at the level: <sup>a</sup><sub>P</sub> < 0.05; <sup>b</sup><sub>P</sub> < 0.02; <sup>c</sup><sub>P</sub> < 0.01

Induced aflatoxicosis in rabbits caused reduction in MCV. Enhanced rate of hemolysis during aflatoxicosis might cause release of smaller sized premature cells in the blood stream from bone marrow.

In summary, erythrocytes and its related parameters are affected when young rabbits were fed with aflatoxin contaminated feed (15 mg/kg) for 60 days. Aflatoxin treatments resulted in significant reduction of erythrocytes, hemoglobin and PCV. Alterations in MCV, MCH and MCHC were also recorded. A time dependent response was observed suggesting cumulative toxicity during aflatoxicosis.

**Acknowledgements** Authors are thankful to Prof. H.C. Dube for his constant encouragement. Financial assistance from Gujarat Council of Science and Technology is gratefully acknowledged.

## REFERENCES

- Bortell R, Asquith RL, Edds GT, Simpson CF, Aller WW (1983) Acute experimentally induced aflatoxicosis in the weanling pony. *Amer J Vet Res* 44: 2110-2214.
- Brucato M, Sundlof SF, Bell JU, Edds GT (1986) Aflatoxin B1 toxicosis in dairy calves pretreated with selenium-vitamin E. *Amer J Vet Res* 47: 179-183.
- Busby WF, Wogan GN (1984) Aflatoxins. In: Searle CE (ed) *Chemical Carcinogens*, ACS Monograph 182, Amer Chem Soc, Washington DC pp. 945-1136.
- Clark JD, Jain AV, Hatch RC, Mahaffey EA (1980) Experimentally induced chronic aflatoxicosis in rabbits. *Amer J Vet Res* 41: 1841-1845.
- Clark JD, Hatch RC, Miller DM, Jain AV (1984) Caprine aflatoxicosis: Experimental disease and clinical pathologic changes. *Amer J Vet Res* 45: 1132-1135.
- Cysewski SJ, Pier AC, Engstrom GW, Richard JL, Dougherty RW, Thurston JR (1968) Clinical pathologic features of acute aflatoxicosis of swine. *Amer J Vet Res* 29: 1577-1590.
- Diener UL, Davis ND (1966) Aflatoxin production by isolates of Aspergillus flavus. *Phytopathology* 56: 1390-1393.
- Harvey RB, Clark DE, Huff WE, Kubena LF, Corrier DE, Phillips TD (1988) Suppression of serum iron-binding capacity and bone marrow cellularity in pigs fed aflatoxin. *Bull Environ Contam Toxicol* 40: 576-583.
- Huff WE, Kubena LF, Harvey RB, Corrier DE, Mollenhauer HH (1986) Progression of aflatoxicosis in broiler chickens. *Poultry Sci* 65: 1891-1899.
- Huff WE, Harvey RB, Kubena LF, Rottinghaus GE (1988) Toxic synergism between aflatoxin and T-2 toxin in broiler chickens. *Poultry Sci* 67: 1418-1423.
- Krishnamachari KAVR, Bhat RV, Nagarajan V, Tilak TBG (1975) Investigation into an outbreak of hepatitis in parts of western India. *Indian J Med Res* 63: 1036-1048.

- Mukherjee KL (1988) Medical Laboratory Technology. Tata McGraw-Hill Publishing Company Ltd., New Delhi.
- Nabney J, Nesbitt BF (1965) A spectrophotometric method for determining the aflatoxins. *Analyst* 90: 155-160.
- Panda PC, Murti AS, Murthy VS, Murti IAS (1975) Effect of aflatoxin on the hematological picture of albino rats and guinea pigs. *Indian J Exp Biol.* 13: 569-570.
- Patterson DSP (1983) Aflatoxicosis in farm animals. *Vet Res Comm* 7: 135-140.
- Ranjan KS (1987) Studies on effect of aflatoxin infested meal on some laboratory animals. Ph.D. thesis, Bhagalpur University, Bhagalpur, India.
- Ray AC, Abbitt B, Cotter SR, Murphy MJ, Reagor JC, Robinson RM, West JE, Whitford HW (1986) Bovine abortion and death associated with consumption of aflatoxin-contaminated peanuts. *J Amer Vet Med Assoc* 188: 1187-1188.
- Reddy RV, Sharma RP (1989) Effects of aflatoxin B1 on murine lymphocytic functions. *Toxicology* 54: 31-44.
- Reddy RV, Taylor MJ, Sharma RP (1987) Studies on immune function of CD-1 mice exposed to aflatoxin B1. *Toxicology* 43: 123-132.
- Shank RC, Wogan GN, Gibson JB, Nondasuta A (1972) Dietary aflatoxins and human liver cancer. II. Aflatoxins in market foods and foodstuffs of Thailand and Hong Kong. *Fd Cosmet Toxicol* 10: 61-69.
- Stoloff L (1977) Aflatoxin - an overview. In: Rodricks JV, Hasseltine CW, Mehlman MA (eds) *Mycotoxins in Human and Animal Health*, Pathotox Publishers, Park Forest South 1, pp. 16-28.
- Verma RJ, Raval PJ, Dube HC (1991) Effect of aflatoxin on liver and blood cells of rats. *Indian J Microbiol* 31: 87-89.
- Verma RJ, Raval PJ (1991) Cytotoxicity of aflatoxin on red blood corpuscles. *Bull Environ Contam Toxicol* 47: 428-432.
- Wyatt RD, Neathery MW, Moos WH, Miller WJ, Gentry RP, Ware GO (1985) Effects of dietary aflatoxin and zinc on enzymes and other blood constituents in dairy calves. *J Dairy Sci* 68: 437-442.

Received March 1, 1991; accepted June 30, 1992.