STUDIES OF HEMATOLOGICAL ALTERATIONS IN RABBITS DURING AFLATOXICOSIS

A. S. Kolhe*, S.B.Chaudhari** and R.J.Verma***
*Dept. of Zoology, Arts and Sciences college, Bhalod, Dist. Jalgaon (MS)-425304
** Dept. of Zoology, Institute of Sciences, Mumbai-32
***Dept. of Zoology, University School of Sciences, Gujarat University,Ahmedabad-9(Guj.)

ABSTRACT :

Aflatoxins are a secondary toxic fungal metabolites which commonly grow on human food and animal feeds. To study effects of aflatoxin, adult in bread rabbits were fed a diet containing 7.5 mg aflatoxin / kg feed for 90 days. A time dependent response was observed suggesting cumulative toxicity during aflatoxicosis.For haematological studies, blood samples from ear-pinna of rabbits were collected on 0, 7, 15, 30, 45, 60, 75 and 90 days of treatment and used. Results revealed that feeding aflatoxin contaminated diet caused a significant reduction In erythrocytes count and haemoglobin content. The decreases in RBC count and haemoglobin were continuous and time dependant. But the % reticulocyte count registered a time dependent increase during aflatoxicosis. Significant increase was recorded on 15th day of treatment and thereafter. Morphological alterations included Change in size of the erythrocytes. The number of small sized cells while the number of medium cells increased aflatoxicosis. Decreased.During No definite trend was evident in big cells. Initial Increase in number of big cells (7 and 15 days) was followed with a decrease. Much decrease in counts was noted on 30th and 45th day of treatment. Also average sized cells showed Increase except at 45th day of treatment where a decrease was recorded. Blood samples were also examined for total and differential counts of leucocytes. An Initial upsurge in total count was followed with time dependent significant decrease. The highest share of neutrophils and lymphocytes were accounted during differential counts of leucocytes. An initial upsurge followed by time dependent continuous decrease in number of neutrophils, eosinophils, basophils and monocytes were also noted during aflatoxicosis. But only neutrophils Lymphocytes count showed statistically significant count registered an initial decline with time dependent increase.

Index Terms: Aflatoxin, Aflatoxicosis, Erythrocytes, Rabbits.

I. INTRODUCTION

The presences of aflatoxin in various food/feed stuffs, in exceptionally high concentration [1,20,21,36] pose serious health hazards to human being and animals. Aflatoxin are well known for its hepatotoxic, carcinogenic, mutagenic and teratogenic effects [4,12,34]. Aflatoxin are also reported to be extremely cytotoxic on mammalian cells in culture [18,19] as well as on erythrocytes in suspension [35]. Verma and Raval(1992b) [35] reported that consumption of aflatoxin contaminated feed in a dose of 15 mg/kg for 60

days caused significant reduction in erythrocytes count, hemoglobin content and packed cell volume(P.C.V.). Since the count of hemoglobin containing erythrocytes responsible for transport of gases is strictly monitored [17,32] the body may respond to the changes by liberating more immature erythrocytes to the blood stream. Due to lacking of information the present investigation was undertaken to monitor changes in erythrocytes with even a lower concentration of aflatoxin for 90 days in rabbits.

Leucocytes. the mobile units of body's protective system. tissue macrophage system and the lymphoid tissue combating the infectious and toxic agents of the body. Are formed in the bone marrow (granulocytes and monocytes) and partially in the lymphoid tissues (lymphocytes and plasma cells). After formation. they are released into the blood stream through which it reaches to the different parts of the body where they are used. The granulocytes and the monocytes protects the body against foreign invading organisms by ingesting them in the process of phagocytosis. one function of lymphocyte is also the same, to destroy the invading organisms, a part of the immune system. Conflicting results of total and differential counts of leucocytes during aflatoxicosis have been reported by many investigators [7,15,28,13]. Thrombocytes are especially important for repair of minute breaks in capillaries and other small vessels. Indeed, platelets can aggregate to fill such ruptures without actually causing clot. Increased platelet count insubchronic aflatoxicosis and decreased platelet count and prolongation in prothrombin time has also been reportedin rabbits [5].

II. MATERIAL AND METHODS

Aspergillus parasiticus (NRRL 3240 obtained from IARI.,New Delhi, India) was grown on SMKY liquid medium at 280c for 10 days [8]. Pooled culture filtrate were extracted with analytical grade chloroform and qualitatively analyzed for different types of aflatoxins on TLC plates [31] aflatoxin content was quantified [25] using a shimazu UV160A spectrophotometer .The crude aflatoxin concentrate in chloroform was mixed with feed (7.5mg aflatoxin /kg) and left overnight to allow for chloroform evaporation. The presence of toxin in such toxin mixed ration was ensured by taking random sample and analysis. Feed for control rabbits was prepared in similar way except for addition of toxin.

Young adult New Zealand strain of rabbits (*Oryctolaguscuniculus*) weighing approximately 1.2 kg were maintained under laboratory condition and fed with ration and water *ad-libitum*. Ten rabbits were randomly segregated in two groups and fed with either control or test feed continuously for 90 days.Blood samples collected in EDTA bulbs from the ear pinna of rabbits, at various intervals maintained under laboratory conditions.Erythrocytes and Leucocytes were counted by haemocytomatric method using Neubauer chamber. Hemoglobin content was measured by acid Haematin method [24]. Blood smears prepared after staining the sample with new methylene blue were used for counting reticulocytes[24]. Size of erythrocytes were measured by using micrometer scale [24].Semidried blood smears were fixed in absolute methanol and

stained using field Stain [24] for differential counts of W.B.C. Blood clotting time was recorded by capillary tube method and blood platelets were counted using Neubauer chamber [24].

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

III. RESULTS

Decreased feed and water consumption were the first signs observed in aflatoxin fed rabbits. Treated rabbits exhibit melancholy, nervousness, staggering, muscular spasm, hair loss and lethargy. Ingestion of crude aflatoxin appears to reduce the rate of increase in body weight , could be due to decreased in food intake, disturbed anabolic activities and / or increased catabolic activities [22].

Table 1: Effect of feeding an aflatoxin-contaminated diet on erythrocytes count, hemoglobin content and reticulocytes count in rabbits.(Mean ±S.E.M.; n=5)

Days of Treatment (Aflatoxin fed Rabbits)	Blood Parameters of Rabbits		
	Erythrocytes ($X10^6$)	Hemoglobin (g/dl)	Reticulocytes (%)
0 (Control)	6.40 ±0.41	12.40 ±0.84	2.34 ±0.35
7	6.27 ±0.51	6.27 ±0.51	6.27 ±0.51
15	5.87 ±0.49	5.87 ±0.49	5.87 ±0.49
30	5.27 ±0.46	5.27 ±0.46	5.27 ±0.46
45	4.91^a ±0.42	4.91^a ±0.42	4.91^a ±0.42
60	4.61^a ±0.32	4.61^a ±0.32	4.61^a ±0.32
75	4.60^b ±0.42	4.60^b ±0.42	4.60^b ±0.42
90	4.33^c ±0.40	4.33^c ±0.40	4.33^c ±0.40

As compared with control a= $p<0.05$;b= $p<0.02$;c= $p<0.01$;d= $p<0.001$.

Dietary aflatoxicosis caused a time dependent decrease in erythrocytes count and hemoglobin content. Significantly reduced erythrocytes count and hemoglobin content were recorded from 45 and 60 days onwards of the treatment, respectively. Table 1 also shows increased reticulocytes count during aflatoxicosis in rabbits. Significantly higher countwere recorded from 45 days onwards of the treatment.

Table 2 : Effect of feeding an aflatoxin-contaminated diet on size of erythrocytes (X400) in rabbits.(Mean ±S.E.M.; n=5)

Days of Treatment (Aflatoxin fed Rabbits)	Blood Parameters of Rabbits			
	Small sized (less than 2.5µ)	Medium sized (2.5-3.5 µ)	Big sized (more than3.5µ)	Average size
0 (Control)	47 ±5	47 ±5	47 ±5	2.57
7	39 ±5	39 ±5	39 ±5	2.69
15	36 ±4	36 ±4	36 ±4	2.75
30	32 ±4	32 ±4	32 ±4	2.61

45	26^{a} ±5	26^{a} ±5	26^{a} ±5	2.42
60	19^{b} ±4	19^{b} ±4	19^{b} ±4	2.75
75	14^{c} ±4	14^{c} ±4	14^{c} ±4	2.78
90	14^{c} ±4	14^{c} ±4	14^{c} ±4	2.76

As compared with control a= p<0.02;b= p<0.01;c= p<0.001.

Data presented in table 2 shows changed size of erythrocytes during induced aflatoxicosis in rabbits. While number of small sized cells significantly decreased, the count of medium sized cells increased significantly. No definite trend was observed in big sized cells. Initial Increase In number of big sized cells (7 &15 days) was followed with a decrease; much decreased counts were noted on 30 and 45th day of treatment. Also average size of erythrocytes showed Increase except at 45th day where a decrease was observed

Table 3: Effect of feeding aflatoxin contaminated diet on total and differential counts of WBC (White blood corpuscles) in rabbits during aflatoxicosis.(Mean ±S.E.M.; n=5)

Days of Treat-ment	WBC Total ($X10^{3}$Cells/µl)	Neutrophils (%)	Esinophils (%)	BasophiS (%)	Lymphocytes (%)	Monocytes (%)
o	5.98 ± 0.14	46.00 ± 0.21	1.50 ± 0.22	1.75 ± 0.15	49.50 ± 1.05	3.50 ± 0.35
7	6.34 ± 0.11	49.13^{a} ± 0.22	2.50 ± 0.60	2.015 ± 0.55	42.37^{a} ± 0.25	4.00 ± 0.45
15	5.41^{b} ± 0.09	47.27^{a} ± 1.10	2.45 ± 0.58	1.90 ± 0.13	44.78^{d} ± 1.35	3.60 ± 0.50
30	5.09^{a} ± 0.05	43.10^{a} ± 0.13	2.10^{d} ± 0.02	1.75 ± 0.11	49.90 ± 2.45	3.20 ± 0.75
45	4.99^{a} ± 0.05	41.00^{b} ± 1.23	1.85 ± 0.04	1 .68 ± 0.43	52.40 ± 1.52	3.10 ± 0.07
60	4.64^{a} ± 0.06	40.55^{a} ± 0.08	1.50 ± 0.48	1.54 ± 0.42	53.45^{c} ± 0.75	3.00 ± 0.65
75	4.43^{a} ± 0.06	38.75^{a} ± 0.82	1 .40 ± 0.03	1 .45 ± 0.27	55.45^{C} ± 1.75	2.95 ± 0.45
90	4.01^{a} ± 0.04	35.60^{a} ± 1.23	1.20 ± 0.01	1.40 ± 0.15	58.90^{b} ± 1.90	2.90 ± 0.55

Significant at a = P<0.001; b = P<0.01; c = P<0.02; d = P<0.05

Table 3 revealed that induced aflatoxicosis in rabbits cause changes In total and differential counts of leucocytes. An initial upsurge in total WBC count was followed with time dependent significant decrease. The highest share of neutrophils and lymphocytes were accounted during differential counts of leucocytes. An initial upsurge followed by time dependent continuous decrease in number of neutrophils, eosinophils, basophils and monocytes were noted. only neutrophils count showed statistically significant change. Lymphocytes count registered an Initial decline followed with time dependent Increase. The decrease at 7 and 15 days as well as Increase on 60,

75 and 90 days of treatment were significantly different from the controls. Increased total WBC count on 7th day of treatment could be due to increase in neutrophils, eosinophils, basophils and monocytes whereas lymphocytes count showed a slight decline.

Table 4 Effect of feeding aflatoxin contaminated diet on clotting time and platelets count during aflatoxicosis in rabbits.(Mean ±S.E.M.; n=5)

Blood Parameters of Rabbits	days of treatment (Aflatoxin fed Rabbits)						
	0	15	30	45	60	75	90
Clotting time (seconds)	55.20 ± 2.15	125.60[a] ± 2.01	145.20[a] ± 4.02	103.50[a] ± 1.56	36.50[a] ± 1 .11	73.40[b] ± 2.94	90.40[a] ± 0.67
Platelets (x 10^5 cells/ µl)	4.06 ± 0.16	1.70[a] ± 0.13	1 .42[a] ± 0.16	2.75[a] ± 0.14	4.39[d] ± 0.16	3.53[c] ± 0.11	3.00[a] ± 0.06

Significant at a=P<0.001; b = P<0.0l; c=P<0.02; d = P<0.05

Table 4 shows feeding aflatoxin contaminated diet caused changes in clotting time and platelets count. Results shown that except on 60th day of treatment clotting time increased during aflatoxicosis and the platelets count show significant' decrease. except for a rise noted on 60th day.

IV. DISCUSSION

The present results clearly indicate a decrease in erythrocytes count and hemoglobin concentration. Suggesting occurrence of cumulative toxicity during aflatoxicosis. Ours results corroborates the finding of Panda et al., [26];Clark et. al., [5]; Brucato et al., [3] and Verma and Raval [35]. Decreased erythrocytes count could be due to aflatoxin induced morphological alterations and haemolysis observed in vitro studies [33]. It is presumed that increased haemolysis also occur in vitro because significantly increased concentration of conjugated and unconjugated bilirubins were recorded during aflatoxicosis [23].

Increased reticulocytes count also occur during aflatoxicosis(Table1). Significantly reduction in erythrocytes count could be responsible for enhanced release of immature erythrocytes (reticulocytes) from red bone marrow in the spongy bone, bone of cranium, ribs, sternum, bodies of vertebral and proximal epiphyses of the humerus and femur [17,32]. The presence of comparatively large size of red blood corpuscles in the peripheral circulation also confirms release of immature erythrocytes which are comparatively larger than the normal erythrocytes.

Data obtained from differential counting of 1eucocytes revealed that neutrophils and lymphocytes accounted for the highest share in total WBC count. The results indicate that on 7 days of treatment. there was an increase in WBC count which could be due to increase in neutrophils. At this point lymphocytes count showed a slight decrease. An increase in WBC count due to increase neutrophils and monocytes have been reported earlier [15,14]. Later on the decrease in leucocytes has been reported by Cysewski et.al.,[7];Reddy

et. al.[30],;Reddy and Sharma [29]; It 1s believed that the initial rise in granulocytes may be due to release of reserve leucocytes from bone marrow as a pathological response. Later the decrease in neutrophils is in clinical conformity with the presence of pathological sites/lesions at one or more places. Reduced granulocytic cellularity has been reported in bone marrow of pigs fed with aflatoxin contaminated feed [14] which could be due to reduced protein synthesis. The increased lymphocytes count in later part. Is suggestive of increased intensity of cell-mediated immunological response [2]. Time dependent decline in WBC count indicates occurrence of cumulative toxicity. Further a decrease in granulocytes and an increase in lymphocytes revealed differential effects of aflatoxin in bone marrow and lymph nodes. The respective sites for their production and release. Aflatoxins are the hepatotoxins and depresses plasma proteins and clotting factors [6]. Cilnico-pathologlcal evaluation of blood coagulation has been limited to studies in dogs [11] in chicken [10,9]. From present investigation it is clear that the blood coagulation is affected during aflatoxicosis. Aflatoxicosis with hemorrhage Clotting factors has been reported in several species. have been studied in chickens [10,9] and dogs [11]. Chickens developed prolonged APTTs. PTs and clot reaction times with no decrease in platelet counts. But here in this experiment we are getting decreased platelet count the reason for this decrease might be due to the abnormality that causes aplasia of bone marrow and cytotoxicity. Acquired platelet dysfunction. secondary to hyperfibrinolysis was reported in human patients with liver disease [16,27]

It can be concluded from the above studies that feeding aflatoxin contaminated diet cause alterations in hematological parameters of rabbits during aflatoxicosis.

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