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Aflatoxin B_1 Induced Carcinogenicity in Wistar Rats: Clinical Signs and Growth Performance

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Abstract

Aflatoxin B_1 (AFB_1), a secondary metabolites is produced by Aspergillus flavus and A. parasiticus. The sequential toxic effect of AFB_1 in Wistar male and female rats was investigated on clinical signs and growth rate in male and female Wistar rats, which were continuously administered with purified AFB_1 in feed at low doses (0.0, 0.2 and 0.4 ppm) at 10 week intervals up to 70 weeks. The clinical signs, body weight and body weight gain showed a significant dose, duration and sex dependent decrease in body weight, relative body weight and body weight gain due to long term AFB_1 induced toxicity. Mortality rate was up to 7.77%. The clinical signs and growth rate parameters revealed a good indicator/ marker for the long term toxic effect of aflatoxin B_1 in Wistar rats

Key words: *Aflatoxin B_1, Growth performance, Wistar rats,*

Introduction

Aflatoxin B_1 (AFB_1) is produced as a secondary metabolites by the mould *Aspergillus flavus* and *A. parasiticus* (Ahamad *et al.,* 2006). When acute aflatoxicosis occurs, dramatic signs of illness become apparent or it results in death of affected individuals. Chronic intake may not result in obvious clinical abnormalities. But depressed body condition or production rate, impaired resistance to infectious agents are the general signs of chronic aflatoxicosis (Huwing *et al*., 2001) It is well known that AFB_1 is toxic substance for animals and human health and it originates from animal products (such as meat, milk, egg, cheese, etc.) (Ramos and Hernandez., 1997). Very little data have been published on mid- or long-term feeding studies with AFB_1 with respect to clinical pathology in rats. The present investigation was undertaken to assess the toxic effect on repeated dose with low levels of AFB_1 feed for a long duration (70 weeks) in Wistar male and female rats on clinical signs and growth performance.

Materials and Methods

Production of AFB_1

Aflatoxin B_1 was produced in rice and broken maize by following the method of Ahamad *et al*. (2006). AFB_l was produced using solid media in maize, rice and low salt synthetic liquid medium, by following the method of Reddy *et al.* (1971).

Preparation of Experimental Feeds

As mentioned in the Table 1, the experimental diets were prepared weekly

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once. The standard basal diet tested free of mycotoxins (AFB_1 and ochratoxin A) was used for the preparation of experimental diets for the rats. The known quantity of purified AFB_1 dissolved in chloroform was used and sprayed over the basal diet and mixed thoroughly so as to achieve a concentration of 0, 200 or 400 µg aflatoxin B_1 / kg feed in experimental diet. For control diet only the equivalent volume of chloroform was used for mixing in basal diet. To confirm uniform distribution of AFB_1 at desired levels in experimental diets, aliquots were tested by TLC and spectrophotometrically.

Experimental Animals and Experimental Design

For the study, 540 weaned Wistar male and female rats of 3-4 weeks of age were procured from the Laboratory Animal Resource (LAR) Section of the Indian Veterinary Research Institute, Izatnagar After an acclimatization period of 1 week, the rats were weighed and randomly divided into six groups of 90 rats each. The rats in control groups (I A and I B) were given basal ration, those in Gr. II A & B and III A & B were fed with basal ration feed mixed with 200 and 400 µg AFB_1 / kg feed. Reasons for choosing the Wistar rat as a model were its high susceptibility for chronic toxicity, low rates of spontaneous malformations, convenient size, genetic stability and easy handling. Following allocation, the animals were marked with picric acid solution for individual identification. Animal room temperature and relative humidity were set at 21 ±2oC and 50 ±10% respectively and lighting was controlled to give 12 h light and 12 h darkness cycles. The rats were housed in polypropylene cages and rice husk was used as the bedding material. Rats were caged (6 rats per cage up to 10 weeks and 3 rats per cage from 11 to 70 weeks) separately throughout the experiment. Throughout the study, every cage was properly marked according to group, treatment schedule and animal numbers. All the animals had free access to standard laboratory animal diet tested negative for AFB_1 and clean *ad libitum* water until the day of sacrifice. The rats were observed daily in the morning and evening. In deciding the design of experiment, guidance of the Organization for Economic Co-operation and Development (OECD) guidance notes for analysis of chronic toxicity and carcinogenicity (ENV/JM/MO NO (2000) 19) was followed.

Parameters of the Study

Clinical Signs Observation

The experimental rats including the controls were observed daily twice and clinical manifestations, if any, were recorded throughout the experimental period. The mortality, if any, in different groups were recorded to find out the mortality pattern.

Growth Rate Performance

Mean body weights of experimental rats were recorded every 10 week up to 70th week and body weight gains were also calculated. Relative body weight was also calculated for treatment, dose and sex by comparing with respective control group.

Statistical Analyses

The data obtained in the experiments were subjected to statistical analyses following three way ANOVA as described by Snedecor and Cochran (1989) and the means were tested in three way ANOVA using Duncan's test to study the effects of treatment and duration and sex.

Results

Clinical signs

Signs of chronic aflatoxicosis appeared in the two treated (0.200 and 0.400 ppm AFB_1) groups of rats early in the experiment. The clinical signs were exhibited by 0.4 ppm group male rats from 4th week of intoxication whereas in female rats the clinical signs were started appearing after 13th week post intoxication. The clinical signs like variable degrees of anorexia, increased thirst, growth depression, restlessness, intermittent diarrhoea and even death in few cases were observed. These signs were more pronounced in male rats than in female rats. The clinical signs increased in intenstiy with duration of AFB_1 feeding. The rats after 50th week, became very weak, dull and unthrifty with partial loss of hairs and manifested ducked up appearance, respiratory distress (difficulty breathing), polydypsia and watery diarrhea with foul smell. Both the toxin group rats revealed corneal opacity (30%), conjunctivitis with mucus discharge (40%) and rough hair coat with piloerrection.

Mortality

During overall experimental period, 2.5% (2 out of 80 rats), 1.25% (one out of 80 rats), 7.5% (6 out of 80 rats), 2.5% (2 out of 80 rats), 8.75% (7 out of 80 rats) and 3.75% (3 out of 80 rats) mortality was recorded in Gr. IA, IB, IIA, IIB, IIIA and IIIB respectively. The mortality was recorded at 228 and 301th day in Gr. IA, 219th day in Gr. IB, 173, 229, 305, 382, 453 and 471st day in Gr. IIA, 229 and 405th day in Gr. IIB, 132, 179, 229, 259, 368, 375 and 402nd day in Gr. IIIA and 229, 312 and 386th day in Gr. IIIB. Thus out of 21 deaths, 15 were encountered in males and 6 in female rats. Further, 8 and 10 rats died in 200 and 400 ppb groups, respectively as against 3 deaths in control group.

Body weight

The mean values of body weights at 10 week intervals are presented in Table 1. The control group rats (both males and females) showed progressive increase in their body weight (33.12 g at 0 week and 395.74 g at 70th week). The body weights in treatment groups, however, increased up to 30th week (33.14 to 308.11 g in 0.2 ppm group and 33.21 to 284.33 g in 0.4 ppm group) and thereafter, decreased gradually to become 277.78 g in group II and 240.43 g in group III at 70th week post intoxication. The overall mean body weights in treatment groups irrespective of sex were significantly lower (220.45 in group II and 201.37 g in group III) than in the control ones (252.68 g).The effect of doses of AFB_1 on body weights of males and females was more severe in rats of higher dose group (202.08 in males and 200.68 g in females) than in the rats of low dose groups (224.84 g in males and 216.06 g in females) at 70th week.

Table 1: Effect on Body Weight in AFB_1 Treated Rats (Mean ±S.E)

Interval in week	Interaction between treatment, sex and duration											
	Treatment : Male			Treatment: Female			Sex		Treatment			Duration
	Gr IA	Gr IIA	Gr IIIA	Gr IB	Gr IIB	Gr IIIB	Male	Female	Gr I	Gr II	Gr III	
0 day	35.61 ±0.25^{b}	35.98 ±0.25^{b}	36.03 ±0.21^{b}	30.64 ±0.24^{a}	30.29 ±0.16^{a}	30.4 ±0.17^{a}	35.87 0.14^{P}	30.44 0.11^{P}	33.12 ±0.25^{A}	33.14 ±0.26^{A}	33.21 ±0.25^{A}	33.16 ±0.15^{A}
10	191.86 ±0.34^{f}	175 ±0.43^{e}	165.55 ±0.50^{c}	170.94 ±0.31^{d}	189.6 ±0.47^{f}	169.95 ±0.39^{d}	177.47 ±0.75^{Q}	176.83 ±0.63^{Q}	181.4 0.86^{C}	182.3 ±0.66^{C}	167.75 ±0.36^{B}	177.15 ±0.49^{B}
20	339.25 0.29^{v}	306.05 ±0.75^{r}	280.8 ±0.55^{n}	293.4 0.83^{q}	279.95 ±0.65^{m}	269.6 ±0.69^{kl}	308.97 ±1.69^{T}	280.99 ±0.8^{R}	316.33 ±1.99^{K}	293.09 ±1.22^{H}	275.12 ±0.65^{FG}	294.94 ±1.16^{D}
30	369.02 0.37^{v}	320.84 ±0.86^{t}	287.46 ±0.27^{op}	319.95 ±0.51^{t}	295.37 ±0.45^{q}	281.3 ±0.74	326.24 ±2.55^{V}	298.9 ±1.25^{S}	344.49 ±2.27^{L}	308.11 ±1.27^{JK}	284.33 ±0.49^{G}	312.49 ±1.59^{D}
40	384.02 ±0.68^{w}	314.27 ±1.06^{s}	276.4 0.35^{m}	327.4 ±0.33^{t}	295.44 ±0.49^{q}	276.84 ±0.63^{m}	325.99 ±3.77	299.92 ±1.76^{S}	355.71 ±2.9^{M}	304.86 ±1.13^{IJ}	276.63 ±0.36^{FG}	312.82 ±2.2^{D}
50	398.97 ±0.58^{w}	303.08 ±0.72^{r}	248.67 ±0.55^{t}	337.32 ±0.74^{u}	292.44 ±0.39^{q}	267.13 ±0.39^{k}	318.9 ±5.97^{U}	298.96 ±2.75^{S}	368.15 ±3.59^{N}	297.69 ±0.74^{HI}	258.28 ±1.14^{E}	308.75 ±3.31^{E}
60	418.71 ±0.46^{x}	290.63 ±0.77^{pq}	290.04 ±0.48^{pq}	346.77 1.24^{v}	290.63 ±0.77^{pq}	240.49 ±0.53^{h}	334.21 ±6.9^{N}	295.36 ±6.35^{S}	382.09 ±4.94^{O}	290.63 ±0.54^{H}	266.25 ±3.55^{E}	314.91 ±4.52^{F}
70	432.98 ±0.53^{x}	271.02 ±0.35^{l}	219.34 ±1.06^{g}	360.58 0.56^{v}	284.54 ±1.05^{no}	257.56 ±0.76^{j}	316.27 ±13.67^{T}	303.28 ±6.35^{S}	395.74 ±6.22^{P}	277.78 ±1.33^{F}	240.43 ±3.65^{D}	309.5 ±7.33^{E}
Overall	271.66 ±6.84^{A}	224.84 5.35^{B}	202.08 ±4.8^{C}	233.79 ±5.76^{A}	216.06 ±5.02^{B}	200.68 ±4.66^{C}	233.25 ±3.42**	216.92 ±3.01	252.68 ±4.52^{Z}	220.45 ±3.67^{Y}	201.37 ±3.34^{X}	225.05 ±2.28

Gr.I : 0.0 ppm AFB_1; Gr. II : 0.2 ppm AFB_1; Gr.III : 0.4 ppm AFB_1; Different superscripts within the blocks differ significantly (P<0.01).

Body Weight Gains

The mean values of body weight gains at 10 week intervals are presented in Table 2. The overall mean body weight gain in males differed significantly ($P<0.01$) between the treatment groups, being lower in 0.4 ppm group (34.28 g vs 45.18 g in 0.2 ppm group), but it did not differ significantly in females (38.79 g in 0.4 ppm and 40.88 g in 0.2 ppm group). However, in both the sexes, AFB_1 treatment caused significantly lower weight gains than in their respective controls. The overall effects of AFB_1 treatment on body weight gains irrespective of sexes showed similar trend i.e. significantly low (36.56 g) in 0.4 ppm followed by 43.03 g in 0.2 ppm as against 53.96 g in control group.

Relative Body Weight

The mean values of relative body weights at 10 week intervals are presented in Table 3. The overall, the relative body weight of different treatment groups in male rat was 100, 87.30 and 79.76% in control, 0.200 and 0.400 ppm AFB_1 groups respectively. The over all relative body weight of different sexes were 85.86 and 92.78 % in male and female when compared with its respective control group. The overall toxin treated group showed duration dependent toxicity on relative body weight from 0 to 70 weeks as100, 97.79, 93.04, 90.70, 90.70, 87.88, 83.69, 82.20, 78.23% when it is compared with respective their control group.

Based on different treatment and durations the overall mean body weight of control and treated groups (0.200 and 0.400 ppm AFB_1) showed a significant reduction ($P<0.01$) in overall treatment and duration. The relative body weight of different treatment groups at 70 week was 100, 62.49 and 50.32% in control, 0.200 and 0.400 ppm AFB_1 groups respectively . The overall relative body weight of different treatment group was 100, 84.18 and 75.30% in control, 0.200 and 0.400 ppm AFB_1 treated groups respectively. Similarly, the overall body weight gain showed a significant reduction in different treatment groups and duration where as the overall relative body weight gain of different treatment group was 100, 75.22 and 64.94%.

Discussion

The goal of our present experiment is to study the possible effects of long term repeated low dose levels of clinical toxicity of AFB_1 in male Wistar rats. The biological effects of aflatoxins observed clinically can be categorized in two types such as short time and long time toxic effects depending on dose level and frequency of exposure to AFB_1. Short time toxic effects include acute toxicity with clinical evidence of hepatic damage and nervous signs including sudden death. The early findings were also studied with higher doses and short duration by Parenti *et al.* (1975), Clark *et al.* (1980), Abdel-Hamid *et al.* (1990) and Sahoo (1991). Wangikar (2003) observed rough hair coat and diarrhea in pregnant rats. Nervous manifestations like restlessness and dullness observed in the present study was also reported by Ikegowuma (1983) who detected AFB_1 in brain tissues.

Table 2: Effect on body weight gain in AFB_1 treated rats (Mean ±S.E)

Interval In week	Interaction between treatment, sex and duration											
	Treatment : Male			Treatment: Female			Sex		Treatment			Duration
	Gr I A	Gr IIA	Gr IIIA	Gr IB	Gr IIB	Gr IIIB	Male	Female	Gr I	Gr II	Gr III	
0	0.00 ±0.00^{f}	0.00 ±0.00^{f}	0.00 ±0.00^{f}	0.00 ±0.00^{f}	0.00 ±0.00^{f}	0.00 ±0.00^{f}	0.00 ±0.00^{T}	0.00 ±0.00^{T}	0.00 ±0.00^{E}	0.00 ±0.00^{E}	0.00 ±0.00^{E}	0.00 ±0.00^{C}
10	156.35 ±0.46^{S}	140.8 ±0.6^{q}	132.1 ±0.72^{p}	139.38 ±0.44^{p}	156.97 ±0.62^{s}	136.33 ±0.55^{p}	143.08 ±0.74^{Y}	144.23 ±0.67^{Y}	147.87 ±0.74^{L}	148.88 ±0.77^{L}	134.21 ±0.48^{K}	143.66 ±0.5^{E}
20	147.12 ±0.49^{r}	132.69 ±1.03^{p}	99.43 ±1.25^{n}	119.11 ±1.19^{o}	97.18 ±1.17^{n}	103.46 ±0.95^{n}	126.67 ±1.49^{V}	106.63 ±0.9^{W}	133.12 ±1.35^{K}	115.06 ±1.7^{J}	101.47 ±0.8^{I}	116.63 ±1^{D}
30	29.5 ±0.46^{m}	17.76 ±1.47	6.61 ±0.52^{h}	19.02 ±2.33^{k}	3.65 ±2.01^{g}	3.27 ±1.28^{o}	18.09 ±0.89^{V}	8.67 ±1.23^{U}	24.26 ±1.27^{H}	10.7 ±1.4^{F}	4.91 ±0.71^{EF}	13.35 ±0.8^{C}
40	15.04 ±0.81^{k}	-5.03 ±1.12^{e}	-17.02 ±0.78^{c}	-4.35 ±3.07^{f}	-18.03 ±1.96^{c}	-11.94 ±0.75	-2.01 ±1.23^{S}	-11.39 ±1.32^{Q}	5.34 ±1.86^{EG}	-11.53 ±1.31^{D}	-14.4 ±0.6^{B}	-6.75 ±0.94^{B}
50	15.43 ±0.78^{k}	-19.52 ±1.3^{c}	-47.03 ±0.84^{b}	-6.91 ±4.56^{e}	-19.2 ±1.54^{c}	-12.87 ±1.23	-16.2 ±2.51^{P}	-12.99 ±1.71^{Q}	4.26 ±2.64^{EG}	-19.36 ±1^{BC}	-29.25 ±2.15^{A}	-14.57 ±1.51
60	20.55 ±0.96^{l}	-40.75 ±2.69^{b}	-4.13 ±0.72	-15.07 ±5.02^{c}	-13.61 ±1.14^{c}	-40.97 ±3.79^{b}	-7.75 ±3.01^{R}	-22.55 ±2.57^{P}	2.42 ±3.54^{E}	-27.18 ±2.39^{A}	-21.8 ±3.21^{A}	-15.1 ±2.06^{A}
70	13.66 ±0.99^{jk}	-20.71 ±1.21^{c}	-70.51 ±1.09^{a}	11.44 ±1.29^{ii}	-7.19 ±1.63^{d}	17.26 ±1.23	-22.0 ±5.06^{P}	7.34 ±1.66^{U}	12.52 ±0.83^{G}	-13.95 ±1.57^{BC}	-22.08 ±8.29^{A}	-6.76 ±2.97^{B}
Overall	61.92 ±3.21^{C}	45.18 ±3.37^{B}	34.28 ±3.19^{A}	46.05 ±3.07^{B}	40.88 ±3.31^{AB}	38.79 ±3.04^{A}	47.28 ±1.91**	41.93 ±1.81	53.96 ±2.24^{W}	43.03 ±2.36^{V}	36.56 ±2.2^{U}	44.59 ±1.32

Gr.I : 0.0 ppm AFB_1; Gr. II : 0.2 ppm AFB_1; Gr.III : 0.4 ppm AFB_1; Different superscripts within the blocks differ significantly (P<0.01).

Table 3: Effect on relative body weight in AFB_1 treated rats

Interval in week	Interaction between treatment, sex and duration											
	Treatment : Male			Treatment: Female			Sex		Treatment			Duration (%)
	Gr IA (%)	Gr IIA (%)	Gr IIIA (%)	Gr IB (%)	Gr IIB (%)	Gr IIIB (%)	Male (%)	Female (%)	Gr I (%)	Gr II (%)	Gr III (%)	
0	100	100	100	100	100	100	100	100	100	100	100	100
10	100	91.21	86.29	100	110.92	99.42	92.50	103.44	100	100.55	92.27	97.79
20	100	90.21	82.77	100	95.42	91.91	91.07	95.77	100	92.72	87.03	93.04
30	100	86.94	77.90	100	92.32	87.92	88.41	93.42	100	89.54	82.56	90.70
40	100	81.84	71.98	100	90.24	84.56	84.89	91.72	100	85.63	77.75	87.88
50	100	75.97	62.33	100	86.70	79.19	79.93	88.63	100	80.71	70.11	83.69
60	100	69.41	69.27	100	83.81	69.35	79.82	85.17	100	75.92	69.63	82.20
70	100	62.59	50.66	100	78.92	71.43	73.05	84.11	100	70.13	60.75	78.23
Overall	100	82.77	74.39	100	92.42	85.83	85.86	92.78	100	87.30	79.76	89.29

In our study, long term daily exposure of low levels of AFB_1 (0. 2 and 0.4 ppm) with chronic aflatoxicosis up to 70 weeks with reduction of feed efficiency and food conversion, decreased body weight and weight gain were noticed. Symptoms were characterised by anorexia, increased thirst, growth depression, intermittent diarrhoea, dullness and even death particularly in high dose group rats. These clinical signs increased in intensity with dose of AFB_1 and duration and were more pronounced in high dose rats than in lower dose rats. Chronic exposure of a dairy herd to aflatoxin contaminated corn (120 ppb) resulted in severe herd health problems, including the birth of small, unhealthy calves, diarrhea, acute mastitis, respiratory disorders, prolapsed rectum, hair loss, and reduced feed consumption (Guthrie, 1979). Mortality rate in AFB_1 treated group rats were higher than control group. Among the AFB_1 treated rats, higher mortality was observed in male rats (up to 8.75%). The reduction in body weight and body weight gain in AFB_1 treated rats could be attributed to anorexia which developed during the course of toxicity. Reduction in body weight and body weight gain in chronic aflatoxicosis was also observed in rats by earlier workers (Lancaster *et al.,* 1961; Baldwin and Parker, 1985 and Parthasarathy, 1990).

In the present study, higher mortality rate, reduction in growth rate and relative body weight were recorded in adult males than adult females. Similar findings were recorded by Parthasarathy, (1990) and he higher susceptibility of male rats to AFB_1 toxicity. Wogan and Wogan and Newberne (1967) recorded up to 100% mortality in male rats as compared to 36% in females and it showed role of testosterone in making the male intact rats highly susceptible to AFB_1 toxicity than the castrated males (Righter *et al.,* 1972). However, contrary to it, Fong and Chan (1981), Newberne *et al.* (1964), Newberne (1973) and Newberne and Rogers (1973) did not find adverse effect on body weight gain with chronic aflatoxicosis in rats. This might be due to use of a very low doses (0.005 to 0.1 ppm) and crude form of aflatoxin which was beyond low dose level used more over, it was purified AFB_1 we utilized in the present study.

The impact of AFB_1 treatment affects early growth. The results obtained in this study suggest a high risk for human health because of the possibility of indirect exposure through meat and other animal products. It is concluded that low level of AFB_1 affect the normal physiological phenomena of both male and female rats and causes reduction in growth rate.

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