Aflatoxin B_1 Induced Carcinogenicity in Wistar Rats: 2. Aflatoxin B_1 and Aflatoxin M_1 Residues in Muscle and Serum

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Abstract

The sequential serum toxicity was investigated on toxic residues of aflatoxin B_1 and aflatoxin M_1 in muscle and serum in male and female Wistar rats 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 dose, duration and sex dependent levels of AFB_1 and AFM_1 residues in muscle and serum were recorded at highly significant level. The toxic residues of aflatoxin B_1 and aflatoxin M_1 in muscle and serum revealed a good indicator for the chronic toxic effect of aflatoxin B_1 in Wistar rats

Key words: *Aflatoxin B_1 and Aflatoxin M_1 residues, Serum, Wistar rats*

Introduction

Aflatoxin B_1 (AFB_1) is produced as a secondary metabolites by the mould *Aspergillus flavus* and *A. parasiticus*. Aflatoxins may have also adverse effects on immune responses to infectious agents and some of them are potent carcinogen, hepatotoxic, nephrotoxic and genotoxic (Benkerroum and Tantaoui-Elaraki, 2001; Parlat *et al*., 2001). It can enter human and animal dietary system by indirect and direct contamination. Consumption of AFB_1 in many parts of the world varies from 0- 30, 000 ng/ kg / day. It has been estimated that 25% of the world's crops may be contaminated with mycotoxins and the worldwide contamination of foods and feeds with mycotoxins is a significant problem (Hussein and Brasel, 2001**)**. Since 1994, many countries have developed regulations for aflatoxin, and other mycotoxins in animal feeds and human food but the regulation has varied by country. In developing countries such as India, even 100% of maize samples have been contaminated with aflatoxins in the range of 6,250-15,600 ppb (Krishnamachari *et al*., 1975). 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.) (Madden and Stahr., 1995; Ramos and Hernandez., 1997). Sporadic outbreaks with AFB_1 accompanied by inappetance and vague signs of illness may suggested the presence of hepatotoxin in the ration. Clinical alterations varies with the susceptibility of individual species to AFB_1. 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 residual level on

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repeated dose with low levels of AFB_1 feed for a long duration (70 weeks) in Wistar male and female rats on AFB_1 and its metabolite residues in muscle and serum in Wistar male and female rats.

Materials and methods

Production and extraction of aflatoxin B_1

AFB_l was produced using low salt synthetic liquid medium, pH 4.5 prepared by the method of Reddy *et al.* (1971) and estimation of AFB_1 was done by thin layer chromatography and spectrophotometry.

Preparation of experimental feeds

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 rats of 3-4 weeks of age procured from the Laboratory Animal Resource (LAR) Section of the Institute. After an acclimatization period of 1 week, the rats were weighed and were randomly divided into three groups of 90 rats each. The rats in control group was given basal ration, those in Gr. II and III basal ration feed mixed with 200 and 400 µg AFB_1 / kg feed respectively. 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±2^oC 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

Estimation of the AFB_1 and AFM_1 residues in serum

The toxic residue (AFB_1 and AFM_1) concentrations were estimated in sera and muscle by the HPLC method of Miyata *et al*. (2004) with slight

modification. Serum (25 µl) was mixed with 25 µl of nomal saline and 50 µl of ice-cold methanol and then cooled at -20°C for 3 h. The supernatant after centrifugation for 10 min at 15,000 g by a high speed refrigerated centrifuge (Beckman Coulter, USA) was subjected to HPLC (Monroe and Eaton, 1987; Woodall *et al*., 1999). For estimation of toxin residues in skeletal muscle, 1.0 g of muscle was mixed with 2.0 ml of normal saline and homogenised. The homogenate (100 µl) was mixed with 100 µl of normal saline and 400 µl of ethyl acetate. The mixture was centrifuged for 10 min at 15,000 g by a high speed refrigerated centrifuge (Beckman Coulter, USA) after vortexing and the supernatant layer was recovered and dried. The residues were redissolved with 100 µl of normal saline and 100 µl of methanol. HPLC analyses were performed SHIADZU make SCL-10 AV1 with system controller and pump model LC-10 AT VP SHIADZU make RF-10 AXL fluorescence detector. AFB_1 was separated with Phenomenex RPC-18 (250x4.60 mm) column Luna 5 ì C18 (2) using a flow rate of 0.5 ml/min at 40°C. The column was eluted 1.1 mM $HClO_4/NaClO_4$ (pH 3): methanol: tetrahydrofuran (57:38:5). AFB_1 and AFM_1 were detected using the fluorescence setting at an excitation wave lengths of 365 and 361 nm and emission wave lengths of 440 and 421 nm, respectively.

Statistical analyses

The data obtained in the experiments were subjected to statistical analyses following two 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.

Results

Toxic residues of AFB_1 in muscle

The AFB_1 residues in muscles also followed dose and time dependent pattern. Males exhibited high concentration of AFB_1 in muscles than females. The highest mean concentration of AFB_1 in muscles of rats were recorded in Gr. IIIA (0.04±0.01 µg/g) at 70^{th} week. The mean concentration of AFB_1 in muscle (µg/g) was significantly higher (P<0.01) with AFB_1 Gr. I, II and III treatment with 0.00 ± 0.00^{X} 0.014 ± 0.001^{Y} 0.021 ± 0.002^{Z} µg/g in respectively, Among sex, males (0.017±0.001 µg/g of muscle) showed significantly higher (P<0.01) level than and females (0.006±0.00 µg/g). Similarly, based on duration 0, 10, 20, 30, 40, 50, 60 and 70 th week of treatment, the residue level of AFM_1 in muscle was significantly increased as 0.0 ± 0.0, 0.002 ± 0.00^{M}, 0.004 ± 0.001^{M}, 0.006 ± 0.001^{N}, 0.009 ± 0.001^{O}, 0.016 ± 0.002^{P}, 0.021 ± 0.003^{Q} and 0.025 ± 0.003^{R} µg/g respectively. The highest mean concentration of AFM_1 in muscles recorded was 0.084 ± 0.004^{n} µg/g) in Gr. III males at 70^{th} week and lowest mean concentration of AFM_1 in muscle was 0.002 ± 0.00^{ab} µg/g at 10^{th} week in Gr. II females (Table 1).

Table 1: AFB_1 residue in muscle and serum in AFB_1 treated rats (Mean ± S.E.)

Interaction between treatment, sex and duration												
Interval in weeks	**Male**			**Female**			**Sex-wise**		**Treatment-wise**			**Duration**
	Gr I A	**Gr IIA**	**Gr IIIA**	**Gr IB**	**Gr IIB**	**Gr IIIB**	**Male**	**Female**	**Gr I**	**Gr II**	**Gr III**	
Muscle (µg/g)												
0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00±0.00	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{P}	0.00 ± 0.00^{P}	0.00 ± 0.00^{a}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{L}
10	0.00 ± 0.00^{a}	0.004 ± 0.00^{abc}	0.006 ± 0.001^{abc}	0.00 ± 0.00^{a}	0.002 ± 0.00^{ab}	0.003 ± 0.00^{abc}	0.003 ± 0.00^{PQ}	0.001 ± 0.00^{PQ}	0.00 ± 0.00^{a}	0.003 ± 0.00^{A}	0.004 ± 0.00^{AB}	0.002 ± 0.00^{M}
20	0.00 ± 0.00^{a}	0.007 ± 0.00^{abc}	0.011 ± 0.001^{def}	0.00 ± 0.00^{a}	0.003 ± 0.00^{abcd}	0.003 ± 0.00^{abcd}	0.006 ± 0.001^{PQ}	0.002 ± 0.00^{PQ}	0.00 ± 0.00^{a}	0.005 ± 0.00^{AB}	0.007 ± 0.001^{ABC}	0.004 ± 0.001^{M}
30	0.00 ± 0.00^{a}	0.011 ± 0.001^{def}	0.016 ± 0.00^{efghi}	0.00 ± 0.00^{a}	0.005 ± 0.00^{abcd}	0.007 ± 0.00^{abcd}	0.009 ± 0.001^{PQ}	0.004 ± 0.001^{PQ}	0.00 ± 0.00^{a}	0.008 ± 0.001^{ABC}	0.011 ± 0.001^{ABC}	0.006 ± 0.001^{N}
40	0.00 ± 0.00^{a}	0.016 ± 0.001^{efgh}	0.021 ± 0.001^{hij}	0.00 ± 0.00^{a}	0.008 ± 0.00^{bcde}	0.01 ± 0.00^{bcde}	0.012 ± 0.002^{PQR}	0.006 ± 0.001^{PQ}	0.00 ± 0.00^{a}	0.012 ± 0.001^{ABCD}	0.016 ± 0.001^{BCD}	0.009 ± 0.001^{O}
50	0.00 ± 0.00^{a}	0.025 ± 0.002^{j}	0.042 ± 0.002^{l}	0.00 ± 0.00^{a}	0.011 ± 0.00^{cde}	0.014 ± 0.00^{efgh}	0.023 ± 0.003^{RS}	0.009 ± 0.001^{PQ}	0.00 ± 0.00^{a}	0.018 ± 0.002^{CDE}	0.028 ± 0.003^{EFGH}	0.016 ± 0.002^{P}
60	0.00 ± 0.00^{a}	0.033 ± 0.00^{k}	0.061 ± 0.006^{m}	0.00 ± 0.00^{a}	0.015 ± 0.001^{efghi}	0.018 ± 0.001^{ghij}	0.031 ± 0.005^{ST}	0.011 ± 0.001^{PQR}	0.00 ± 0.00^{a}	0.024 ± 0.002^{DEF}	0.04 ± 0.006^{FGH}	0.021 ± 0.003^{Q}
70	0.00 ± 0.00^{a}	0.046 ± 0.002^{l}	0.084 ± 0.004^{n}	0.00 ± 0.00^{a}	0.019 ± 0.00^{hij}	0.023 ± 0.001^{ij}	0.039 ± 0.005^{T}	0.013 ± 0.001^{QR}	0.00 ± 0.00^{a}	0.032 ± 0.003^{GH}	0.05 ± 0.006^{H}	0.025 ± 0.003^{R}
Overall	0.00 ± 0.00^{G}	0.019 ± 0.002^{I}	0.032 ± 0.003^{J}	0.00 ± 0.00^{G}	0.009 ± 0.00^{H}	0.011 ± 0.001^{H}	0.017±0.001**	0.006±0.00	0.00 ± 0.00^{X}	0.014 ± 0.001^{Y}	0.021 ± 0.002^{Z}	0.012±0.001
Serum (µg/ml)												
0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{P}	0.00 ± 0.00^{P}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{A}	0.00 ± 0.00^{L}
10	0.00 ± 0.00^{a}	0.08 ± 0.00^{a}	0.14 ± 0.01^{a}	0.00 ± 0.00^{a}	0.02 ± 0.00^{a}	0.03 ± 0.00^{a}	0.07 ± 0.01^{P}	0.01 ± 0.00^{P}	0.00 ± 0.00^{A}	0.05 ± 0.01^{A}	0.09 ± 0.01^{A}	0.04 ± 0.01^{L}
20	0.00 ± 0.00^{a}	0.19 ± 0.01^{a}	0.65 ± 0.06^{ab}	0.00 ± 0.00^{a}	0.07 ± 0.00^{ab}	0.10 ± 0.01^{a}	0.28 ± 0.05^{P}	0.06 ± 0.01^{P}	0.00 ± 0.00^{A}	0.13 ± 0.02^{A}	0.38 ± 0.07^{AB}	0.17 ± 0.03^{L}
30	0.00 ± 0.00^{a}	1.20 ± 0.14^{ab}	1.36 ± 0.14^{ab}	0.00 ± 0.00^{a}	0.11 ± 0.00^{a}	0.16 ± 0.01^{a}	0.85 ± 0.13^{P}	0.09 ± 0.01^{P}	0.00 ± 0.00^{A}	0.65 ± 0.14^{ABC}	0.76 ± 0.15^{ABC}	0.47 ± 0.08^{L}
40	0.00 ± 0.00^{a}	2.24 ± 0.15^{bc}	3.87 ± 0.18^{cd}	0.00 ± 0.00^{a}	0.22 ± 0.01^{a}	0.33 ± 0.01^{ab}	2.04 ± 0.30^{PQ}	0.19 ± 0.03^{P}	0.00 ± 0.00^{A}	1.23 ± 0.24^{ABC}	2.10 ± 0.42^{ABC}	1.11 ± 0.19^{M}
50	0.00 ± 0.00^{a}	4.12 ± 0.26^{cde}	5.64 ± 0.38^{de}	0.00 ± 0.00^{a}	0.31 ± 0.01^{ab}	0.50 ± 0.02^{ab}	3.25 ± 0.47^{Q}	0.28 ± 0.04^{P}	0.00 ± 0.00^{A}	2.21 ± 0.46^{ABC}	3.07 ± 0.62^{BC}	1.79 ± 0.31^{N}
60	0.00 ± 0.00^{a}	6.07 ± 0.40^{e}	12.13 ± 0.38^{f}	0.00 ± 0.00^{a}	0.53 ± 0.02^{ab}	0.95 ± 0.06^{ab}	6.07 ± 0.94^{R}	0.49 ± 0.08^{P}	0.00 ± 0.00^{A}	3.30 ± 0.67^{CD}	6.54 ± 1.30^{EF}	3.28 ± 0.59^{O}
70	0.00 ± 0.00^{a}	11.08 ± 0.42^{f}	15.57 ± 1.33^{g}	0.00 ± 0.00^{a}	2.49 ± 0.12^{c}	4.17 ± 1.01^{cde}	8.01 ± 1.07^{R}	2.10 ± 0.39^{PQ}	0.00 ± 0.00^{A}	6.52 ± 0.80^{DE}	9.28 ± 1.34^{F}	4.87 ± 0.62^{P}
Overall	0.00 ± 0.00^{G}	3.59 ± 0.44^{H}	5.31 ± 0.67^{I}	0.00 ± 0.00^{G}	0.63 ± 0.10^{G}	1.02 ± 0.25^{G}	2.91±0.30**	0.55±0.09	0.00 ± 0.00^{X}	2.09 ± 0.25^{Y}	3.12 ± 0.39^{Z}	1.72±0.16

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 ($P<0.01$).

Toxic residues of AFB_1 in serum

The mean concentration of AFB_1 in serum (µg/g) was significantly higher (P<0.01) with AFB_1 Gr. I, II and III treatment with 0.00 ± 0.00^{X}, 2.09 ± 0.25^{Y}, 3.12 ± 0.39^{Z} µg/g in respectively, Among sex, males (2.91±0.30**µg/g of muscle) showed significantly higher (P<0.01) level than and females 0.55±0.09µg/g). Similarly, based on duration 0, 10, 20, 30, 40, 50, 60 and 70 th week of treatment, the residue level of AFM_1 in muscle was significantly increased as 0.0±0.0, 0.04 ± 0.01^{L}, 0.17 ± 0.03^{L}, 0.47 ± 0.08^{L}, 1.11 ± 0.19^{M}, 1.79 ± 0.31^{N} , 3.28 ± 0.59^{O} and 4.87 ± 0.62^{P} µg/g respectively. The highest mean concentration of AFB_1 in serum recorded 15.57 ± 1.33^{g} µg/g) in Gr. III males at 70^{th} week and lowest mean concentration of AFB_1 in serum was 0.02 ± 0.00^{a} µg/g at 10^{th} week in Gr. II females (Table 1).

Toxic residues of AFM_1 muscle

The mean concentration of AFM_1 in muscle (µg/g) was significantly higher (P<0.01) with AFB_1 Gr. I,II and III treatment with 0.0±0.0, 0.81±0.10 and 1.26±0.14 µg/g in respectively, Among sex, males (1.17±0.11 µg/g of muscle) showed significantly higher (P<0.01) level than and females (0.21±0.22 µg/g). Similarly, based on duration 0, 10, 20, 30, 40, 50, 60 and 70 th week of treatment, the residue level of AFM_1 in muscle was significantly increased as 0.0±0.0, 0.01±0.003, 0.20±0.03, 0.04±0.06, 0.59±0.10, 0.92±0.17, 1.16±0.20 and 1.62±0.23 µg/g respectively. The highest mean concentration of AFM_1 in muscles recorded was 5.51±0.75 µg/g) in Gr. III A at 70^{th} week and lowest mean concentration of AFM_1 in muscle was 0.004±0.001 µg/g at 10^{th} week in Gr. IIB (Table 2).

Toxic residues of AFM_1 in serum

The mean concentration of AFM_1 in serum was significantly increased (P<0.01) with AFB_1 dose I, II and III with 0.0±0.0, 3.73±0.35 and 5.61±0.52 µg/ml respectively. Among sex males showed a highly significant increased level of AFM_1 residues (4.75±0.40 µg/ml) than females (1.43±0.16 µg/ml). Based on duration 0, 10, 20, 30, 40, 50, 60 and 70th week of treatment, the residue level of AFM_1 in serum was significantly increased as 0.0±0.0, 0.12±0.02, 0.55±0.09, 1.74±0.30, 2.60±0.41, 4.21±0.62, 5.44±0.71 and 7.30±0.80 µg/ml respectively from 0 week to 70^{th} week post AFB_1 treatment. The highest mean concentration of AFM_1 in serum was 21.40±1.48 µg/ml at 70^{th} week in Gr. IIIA and the lowest mean concentration of AFM_1 in serum was 0.03±0.001 µg/ml in Gr. IIB at 10^{th} week (Table 2).

Table 2: AFM_1 residue in muscle and serum in AFB_1 treated rats (Mean ± S.E.)

Interaction between treatment, sex and duration												
Interval in weeks	**Male**			**Female**			**Sex-wise**		**Treatment-wise**			**Interval in weeks**
	Gr IA	**Gr IIA**	**Gr IIIA**	**Gr IB**	**Gr IIB**	**Gr IIIB**	**Male**	**Female**	**Gr I**	**Gr II**	**Gr III**	
Muscle (µg/g)												
0	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{P}	0.00±0.00^{P}	0.00±0.00^{a}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{L}
10	0.00±0.00^{a}	0.04±0.01^{a}	0.04±0.01^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.01±0.00^{a}	0.03±0.01^{P}	0.00±0.00^{P}	0.00±0.00^{a}	0.02±0.01^{AB}	0.02±0.01^{AB}	0.01±0.00^{L}
20	0.00±0.00^{a}	0.36±0.01^{abc}	0.65±0.06^{abc}	0.00±0.00^{a}	0.06±0.00^{ab}	0.11±0.01^{abc}	0.34±0.05^{PQ}	0.05±0.01^{P}	0.00±0.00^{a}	0.21±0.04^{ABC}	0.38±0.07^{ABC}	0.20±0.03^{LM}
30	0.00±0.00^{a}	0.75±0.04^{abc}	1.17±0.14^{cd}	0.00±0.00^{a}	0.10±0.00^{ab}	0.18±0.01^{abc}	0.64±0.10^{PQ}	0.09±0.01^{P}	0.00±0.00^{a}	0.42±0.08^{ABC}	0.68±0.13^{ABCD}	0.37±0.06^{MN}
40	0.00±0.00^{a}	1.01±0.02^{abc}	2.15±0.13^{ef}	0.00±0.00^{a}	0.13±0.01^{ab}	0.24±0.01^{abc}	1.05±0.17^{QR}	0.12±0.02^{PQ}	0.00±0.00^{a}	0.57±0.10^{ABCD}	1.19±0.23^{BCDE}	0.59±0.10^{O}
50	0.00±0.00^{a}	1.88±0.26^{de}	2.97±0.38^{fg}	0.00±0.00^{a}	0.20±0.01^{ab}	0.38±0.02^{abc}	1.62±0.27^{RS}	0.20±0.03^{PQ}	0.00±0.00^{a}	1.04±0.23^{ABCDE}	1.68±0.35^{D}	0.92±0.17^{P}
60	0.00±0.00^{a}	2.31±0.28^{ef}	3.70±0.38^{g}	0.00±0.00^{a}	0.25±0.02^{abc}	0.67±0.06^{abc}	2.01±0.32ST	0.31±0.06^{PQ}	0.00±0.00^{a}	1.28±0.27^{CDE}	2.19±0.40^{F}	1.16±0.20^{P}
70	0.00±0.00^{a}	3.81±0.42^{g}	5.51±0.75^{h}	0.00±0.00^{a}	0.69±0.10^{abc}	1.11±0.14^{bcd}	2.80±0.43^{T}	0.57±0.08^{PQ}	0.00±0.00^{a}	2.16±0.34^{EF}	3.08±0.53^{F}	1.62±0.23^{Q}
Overall	0.00±0.00^{G}	1.42±0.17^{I}	2.15±0.25^{J}	0.00±0.00^{G}	0.22±0.03^{H}	0.39±0.05^{H}	1.17±0.11**	0.20±0.02	0.00±0.00^{L}	0.81±0.10^{M}	1.26±0.14^{N}	0.68±0.06
Serum (µg/ml)												
0	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{P}	0.00±0.00^{P}	0.00±0.00^{P}	0.00±0.00^{a}	0.00±0.00^{A}	0.00±0.00^{A}	0.00±0.00^{L}
10	0.00±0.00^{a}	0.24±0.01^{a}	0.37±0.01^{a}	0.00±0.00^{a}	0.03±0.00^{a}	0.21±0.03^{P}	0.06±0.00^{P}	0.03±0.01^{P}	0.00±0.00^{a}	0.14±0.02^{A}	0.22±0.04^{A}	0.12±0.02^{L}
20	0.00±0.00^{a}	0.89±0.02^{abc}	1.91±0.06^{abc}	0.00±0.00^{a}	0.22±0.00^{a}	0.93±0.15^{PQ}	0.30±0.01^{P}	0.17±0.02^{P}	0.00±0.00^{a}	0.55±0.08^{A}	1.10±0.19^{A}	0.55±0.09^{L}
30	0.00±0.00^{a}	3.76±0.14^{def}	5.87±0.14^{g}	0.00±0.00^{a}	0.31±0.00^{a}	3.21±0.46^{PQR}	0.50±0.01^{P}	0.27±0.04^{P}	0.00±0.00^{a}	2.03±0.40^{AB}	3.19±0.62^{AB}	1.74±0.30^{M}
40	0.00±0.00^{a}	5.06±0.15^{g}	8.37±0.18^{h}	0.00±0.00^{a}	0.67±0.01^{ab}	4.47±0.64^{RS}	1.49±0.01^{R}	0.72±0.11^{P}	0.00±0.00^{a}	2.86±0.51^{AB}	4.93±0.79^{BC}	2.60±0.41^{N}
50	0.00±0.00^{a}	8.53±0.01^{h}	12.37±0.02^{jk}	0.00±0.00^{a}	1.14±0.01^{abc}	6.97±0.96ST	2.78±0.02^{R}	1.35±0.22^{PQR}	0.00±0.00^{a}	4.84±0.85^{BC}	7.58±1.10^{CD}	4.21±0.62^{O}
60	0.00±0.00^{a}	10.39±0.40^{hi}	14.55±0.38^{kl}	0.00±0.00^{a}	2.89±0.02^{cdef}	8.31±1.15^{TU}	4.78±0.15^{R}	2.56±0.37^{PQR}	0.00±0.00^{a}	6.64±0.88^{CD}	9.67±1.14^{D}	5.44±0.71^{P}
70	0.00±0.00^{a}	14.39±0.42^{kl}	21.40±1.47^{m}	0.00±0.00^{a}	4.96±0.10^{efg}	10.74±1.41^{U}	8.55±1.12	4.25±0.60^{QRS}	0.00±0.00^{a}	9.38±0.87^{D}	14.31±1.50^{E}	7.30±0.80^{Q}
Overall	0.00±0.00^{g}	5.94±0.58^{I}	8.59±0.85^{J}	0.00±0.00^{G}	1.57±0.20^{GH}	4.75±0.40^{H}	2.74±0.40**	1.43±0.16	0.00±0.00^{X}	3.73±0.35^{Y}	5.61±0.52^{Z}	3.08±0.23

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).

Discussion

The goal of our present experiment is to study the possible effects of long term repeated low dose levels of serum toxicity of AFB_1 in male and female Wistar rats.

In the present study, highest level of AFB_1 and AFM_1 residues were recorded in males during 70th week of AFB_1 treatment. Adverse effects on animal health and production have been recognized in intensively farmed animals such as poultry, swine and cattle as a consequence of the consumption of high levels of cereals and oilseeds in the diet (Smith and Henderson, 1991; Smith *et al*., 1994). Animals may have varying susceptibilities to mycotoxins depending on physiological, genetic and environmental factors. 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). The currently favored method of measuring human exposure consists of the analysis of body fluids for the presence of aflatoxin derivatives (Makarananda *et al*., 1998; Wild and Pisani, 1998) which is used as biomarkers. Each biochemical process results in derivatives that have a characteristic half-life within the body, and thus the exposure over a period of days, weeks, or months can be assessed. (Williams *et al*., 2004). Measureable quantities of AFB_1 and its metabolic derivatives were detected using high pressure liquid chromatography and fluorescence detection in serum. Chattopadhyay *et al*. (1985) also doubted that quantities of AFB_1 reach many tissues of the organism and the product of metabolism (Mixed function oxidation), specific for each of the tissues which are targets, become consideration. In the present work the overall mean values of AFB_1 residues in serum were observed up to 70th week in AFB_1 treated rats and were dose and time dependent. Free or conjugated aflatoxin B_1 and its hydroxylated metabolite M_1 were reported in muscles (0.01 to 1.19 ng/g tissue) when turkey poults were fed a diet containing AFB_1 (500 ppb) for 18 days (Gregory *et al.,* 1983). The major proportions of aflatoxin residues were reported in the form of water soluble metabolites in blood, tissues and excreta of chicken dosed with 14C AFB_1 (Madee and Chipley, 1973). Residues of AFB_1 (0.015%) and its metabolites, M and B2a (0.005) were also found in muscles of pigs (Furtado *et al.,* 1979). Similarly AFB_1 (80-160 ppb) and AFM_1 (160-240 ppb) were detected in blood and urine samples of pigs. Residues of AFB_1 (40 ppb) and AFM_1 (80 ppb) in blood and AFM_1 alone (80 ppb) in urine of buffaloes and AFB_1 (up to 160 ppb) and AFM_1 (up to 240 ppb) in experimental rat blood (Maryamma *et al.,* 1991); AFB_1 in muscles, (up to 6 ppb) of broiler chicken Arulmozhi *et al.,* 2002), AFB_1 (up to 80 ppb) and AFM_1 (up to 20 ppb) in blood samples, AFM_1 (up to 60 ppb) in urine, (40-80 ppb) in milk of cow in spontaneous cases (Maryamma *et al.,* 1991) were detected. However the analysis of feed samples feeding to cattle contained AFB_1

in hay (4.1 ppb), clover (2.7 ppb) and feed concentrate (5.4 ppb) and further analysis of the their milk revealed AFM_1 was up to 0.258 ppb. The tradition approach to preventing exposure of aflatoxins has been ensured that food consumed have the lowest practical aflatoxin concentration. The route of AFB_1 administration was oral, being the most natural way of getting the AFB_1 through feed. AFM_1, the hydroxylated metabolite of AFB_1, is also equally important in induction of liver tumours in rats (Cullen *et al.,* 1987) at low dose level. Even though, various toxic effects of AFB_1 have been studied with high dose levels either with shorter or longer duration, no literature was available on chronic toxicity with low dose leading to chronic toxicosis.

The impact of AFB_1 treatment affects early growth and at least aspects of humoral immunity and nutrition (Williams *et al*., 2004). At present WHO/ recommended the supplemental foods for under-nourished children should not contained more than 30 ppb AFB_1 in the supplement. This can be shown to be a level having long-term effects on experimental animals but in the light of present knowledge it would appear to be a reasonable level in those developing countries where protein malnutrition is a problem. During continuous administration of AFB_1, steady- state hepatic DNA adduct levels which appear to be linearly related to the concentration of AFB_1 administered chronically (Buss *et al*., 1990). In humans, there is a positive correlation between the amount of AFB_1 ingested and the incidence of liver cancer (Beland and Poirier, 1993). 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. Efforts have continued internationally to establish guidelines to control mycotoxins. Hence the determination of AFB_1 and its metabolites AFB_1 and AFM_1 is required to understand the correlation of exposure of AFB_1 in human beings and quantification of biological markers such as AFB_1 residues in body fluids including serum, etc. alteration in clinical signs. As early markers for the diagnosis of chronic aflatoxicosis will curtail neoplastic disease initiation, promotion and development in individual or carcinogenicity, developmental toxicity and neurotoxicity, teratogenicity in next generation of human beings.

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