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EFFECT OF AFLATOXIN B1 (AFB1) ON VARIOUS BLOOD BIOCHEMICAL & HAEMATOLOGICAL PARAMETERS IN THE EXPERIMENTAL RATS

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ABSTRACT
The present work was designed to reveal the possible effect of aflatoxin B1 on the various blood biochemical parameters in the *Albinus wistar* rats since biochemical parameters are the best indicators of stress situations caused by toxin exposure like pesticides or chemicals. Aflatoxin exposure in humans causes aflatoxicosis involving liver as the principal target organ. Hepatocellular carcinoma is the commonly occurring cancer due to the ingestion of foodstuffs contaminated with aflatoxins. Since the levels of SGOT, SGPT, alkaline phosphatase and bilirubin in the blood stand for the normal functioning of liver, these parameters were taken for assessing the toxic effect of aflatoxin in the experimental animals. Blood urea, creatinine, total cholesterol, and total protein, were assayed for the test and control group of animals in the present investigation. Since *Aspergillus* species of fungi seems to be the predominant contaminant in agricultural commodities especially in stored grains, its toxins like AFB1 was chosen for the present investigation. Many of the biochemical parameters assessed have deviated from their normal values. Alkaline phosphatase activity was increased in the experimental groups that were fed with higher concentration of aflatoxin B1 (14 and 17 mg /kg BW table 12). Whereas the activity of alkaline phosphatase was close to normal and not significantly altered in the experimental rats that were fed with lower levels of aflatoxin B1 (5, 8 and 11mg/kg BW) when compared with the control group. Noticeable differences were observed in haematological parameters of aflatoxin B1 exposed rat after 35 days of exposure in the present study. The values of RBC and WBC showed a significant decrease with increase in concentration of aflatoxin B1 with the control group having the highest values and the group exposed to the highest concentration (17mg/Kg body weight) showed the lowest values.

KEYWORDS: Aflatoxin, Mycotoxins, *Aspergillus flavus*, Biochemical and Haematological parameters.

INTRODUCTION
Rising of farm animals for milk, meat and egg production to feed the ever increasing human population faces a great threat of disease problems in the farm animals. This greatly affects the gross productivity and quality of these animal products. In addition to infectious diseases in farm animals, toxic compounds in feed and forages pose health implications leading to low productivity and inferior quality of animal food products. This signaled the presence of mycotoxins in feeds. Such occurrence of mycotoxins has increased the incidence of acute diseases in farm animals (Forgacs and Carll, 1962). One of such important mycotoxins is aflatoxin produced by *Aspergillus* species of fungi. Aflatoxins are secondary metabolites of *Aspergillus flavus* and *Aspergillus parasiticus* and are known to be toxic to humans and animals. These toxigenic molds are known to contaminate the agricultural products at the field even before the harvest and also at the time of storage. The climatic conditions, temperature and moisture conditions in the field and at storage make amenable for the growth of these toxigenic fungi and toxin production in the contaminated agricultural products. Mycotoxin produced by some strains of *Penicillium* and *Aspergillus* molds that naturally contaminate food and feed under all climatic conditions (Fung and Clark, 2004). A great economic loss is also produced by aflatoxins contamination when attacking different agricultural and dairy products (Cleveland et al., 2003). Aflatoxin contamination in various agricultural commodities and subsequent transmission to animals and humans through feed and food poses great challenge from the perspectives of human and animal health. Mycotoxins contaminations of food and feed stuff are thus considered to lead to economic losses and health concerns both in human and animals (Bryden, 2007; Osweiler, 2000; Pineiro, 2003). *Aspergillus flavus* is the major and common contaminant in agriculture. It is also called storage fungi since it contaminates most of the stored agricultural commodities. *Aspergillus flavus* colonizes

stored grain when factors such as temperature and water activity are optimised (Smith, 1997). The major aflatoxins produced by the *Aspergillus* species are B1, B2, G1 and G2. They are classified based on their chromatographic mobility and fluorescence under UV. Strains of A. flavus and *A. parasiticus* can produce aflatoxins B1, B2, G1, G2 and M1 (Wilson et.al., 1994), contaminating a number of agricultural products such as peanuts, corn and cereal grains (Busby et al., 1984). Aflatoxin B1 is the most potent natural carcinogen known and is usually the major aflatoxin produced by toxigenic strains (Squire, 1981).

Due to the ubiquitous nature of fungi, it has been estimated that approximately 20% of all food products (mainly of plant origin) are contaminated with substantial toxin concentrations (Anklam et al., 2002). Nowadays, the main problem caused by mycotoxins is spoiling agricultural products, which depends on environmental and storage conditions. It is estimated that approximately 25% of the world's crops are contaminated to some extent with mycotoxins (Fink-Gremmels 1999, Mannon et al., 1985, Richard et al., 2003). The contamination of foods and animal feeds with these mycotoxins is a worldwide problem (Ngindu et al., 1982; Smith et al., 1994; Averkieva, 2009). Cereal grains and their products constitute the main energy and protein source for farm animals. When cereal grains are colonized by toxigenic moulds there is a high risk of contamination with mycotoxins. Once the aflatoxin is established in the agricultural products it is very difficult to eliminate and pass on to the animals and humans through feed and food materials because, AFB1 is heat stable, the initial contamination levels could persist to the final product and finally reach the human or animal. Humans, as well as animals, are exposed to mycotoxins in their diets, and this can be considered, in most cases, the route of administration of natural intoxications caused by these compounds. Aflatoxins, particularly in animal feed stuffs, pose serious public health hazards since these are very toxic (Yaling et al., 2008), Human exposure to mycotoxins through consumption of plant-derived foods that are contaminated with toxins, the carry-over of mycotoxins and their metabolites in animal products such as meat and eggs has also been reported by CAST, 2003. In addition to the dietary route of mycotoxin exposure of animals and humans, source of exposure to air borne fungi through bioaerosols from the occupational and residential environments can cause adverse health effects with major public health impact (Douwes et al., 2003). The farming environment is an important source of exposure to airborne fungi (Adhikari et al., 2004b; Halstensen et al., 2004; Krysinska-Traczyk et al., 2007) and can cause several respiratory diseases like asthma, farmer's lung disease (FLD) or rhinitis (Linaker and Smedley, 2002; Reboux et al., 2001). Exposure to air and dust containing toxins can also lead to mycotoxin toxicity (Jarvis, 2002). Carcinogenic and teratogenic effects of mycotoxins have been demonstrated by several workers (Clark and Snedeker, 2006; Pfohl-Leszkowicz and Manderville, 2007; Stark, 2005). Direct consequences of consumption of mycotoxin- contaminated animal feed include: reduced feed intake, feed refusal, poor feed conversion, diminished body weight gain, increased disease incidence (due to immune-suppression) and reduced reproductive capacities (Fink-Gremmels and Malekinejad, 2007; Morgavi and Riley, 2007; Pestka, 2007; Voss and Haschek, 2007) which leads to economic losses (Huwig et al., 2001; Wu, 2006; Wu, 2007).

Aflatoxins entering the animals through feed get metabolized in liver and get converted in to other toxic intermediary compounds and get mixed in the milk, egg and meat. Excretion of aflatoxin in animal products like milk (Abd Alia et al., 2000) and eggs (Aly and Anwer, 2009) has also been reported. Through these animal products man get exposed to the aflatoxin derivatives. The toxic nature of the aflatoxin derivatives have been elucidated by several authors. The binding of these intermediaries to DNA results in the disruption of transcription and abnormal cell proliferation, leading to mutagenesis and carcinogenesis (Guengerich, 2001; Imaoka et al., 1992; Sell et al., 1998). The carcinogenic potential of aflatoxin in a number of animal species have been shown and associated with mycotoxicoses in poultry and in other domestic animals (Quist et al., 2000; Dimitri et al., 1998). Aflatoxin ingestion by animals and higher vertebrates cause diverse health effects and disease called aflatoxicoses (Adegoke and Letuma, 2013). Aflatoxin B1 causes aflatoxicosis, a toxic hepatitis leading to jaundice and, in severe cases, death. Repetitive incidents of this nature have occurred in Kenya during 1981, 2001, 2004 and 2005, India, and Malaysia (Shephard, 2004; Lewis et al., 2005).

The present work was designed to reveal the possible effect of aflatoxin B1 on the various blood biochemical parameters in the *Albinus wistar* rats since biochemical parameters are the best indicators of stress situations caused by toxin exposure like pesticides or chemicals. Aflatoxin exposure in humans causes aflatoxicosis involving liver as the principal target organ. Hepatocellular carcinoma is the commonly occurring cancer due to the ingestion of foodstuffs contaminated with aflatoxins (London et al., 1995). Since the levels of SGOT, SGPT, alkaline phosphatase and bilirubin in the blood stand for the normal functioning of liver, these parameters were taken for assessing the toxic effect of aflatoxin in the experimental animals. Blood urea, creatinine, total cholesterol, and total protein, were assayed for the test and control group of animals in the present investigation. Since *Aspergillus* species of fungi seems to be the predominant contaminant in agricultural commodities especially in stored grains, its toxins like AFB1 was chosen for the present investigation.

MATERIALS AND METHODS

2.1. Animals

Male Wistar rats with an age group ranging between 6-8 weeks with an average body weight 126±2g were selected for the present study. The animals were maintained in polypropylene cages covered by steel wire grid and having bedding of paddy husk. The cages were kept in environmentally controlled rooms with 12-h light / dark cycle. The animals were fed with scientifically prepared and nutritionally balanced rat feed pellets obtained from Hindustan Lever Ltd., and clean drinking water *ad libitum.* The animals were acclimated to the house conditions for 14 days prior to study start.

2.2. Experimental design

The rats were grouped in to six batches each containing10 individuals. The first group served as control group which were fed with aflatoxin free commercial rat feed pellets. From the second to sixth group of animals were fed on diet formulated with different concentrations of Aflatoxin B1 obtained from Sigma Aldrich, USA. The experimental groups of animals were fed with an increasing concentration (5, 8, 11, 14, and 17mg/kg body weight) of Aflatoxin B1 along with the feed. The initial dosage level of Aflatoxin B1 (5mg) was determined at a sub lethal concentration of the toxin and the highest as lethal concentration 17mg/kg BW (LD_{50} value). After oral administration of toxin, rats were observed for changes in spontaneous behavior daily.

2.3. Blood collection

Blood samples were collected in tubes without anticoagulant to separate serum for various biochemical estimations prior to feeding on days 0, 21, 28, 35, 42 and 49 via jugular venipuncture. Plasma and serum were harvested by centrifugation (2500×g for 15 min at 4 ◦C) and stored at −20◦C until analysis.

BLOOD BIOCHEMISTRY

The levels of SGOT, SGPT in the blood serum and plasma concentrations of, total proteins, urea, creatinine, cholesterol and activities of alkaline phosphatase were analyzed. All The biochemical parameters were determined by using a Technicon RA-1000 autoanalyser.

SGOT ASSAY Modified IFCC Method (Bergmeyer *et al.*, 1986)

SGOT (ASAT) catalyzes the transfer of amino group between L-Aspartate and a Ketoglutarate to form Oxaloacetate and Glutamate. The Oxaloacetate formed reacts with NADH in the presence of Malate Dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the SGOT (ASAT) activity in the sample.

SGPT ASSAY (Modified IFCC Method) (Bergmeyer *et al.*, 1986)

SGPT (ALAT) catalyzes the transfer of amino group between L-Alanine and Ketoglutarate to form Pyruvate and Glutamate. The Pyruvate formed reacts with NADH in the presence of Lactate Dehydrogenase to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the SGPT (ALAT) activity in the sample.

ESTIMATION OF ALKALINE PHOSPHATASE PRINCIPLE

ALP at an alkaline pH hydrolyses p-Nitro phenyl phosphate to form p-Nitro phenol and phosphate. The rate of formation of p-Nitro phenol is measured as an increase in absorbance which is proportional to the ALP activity in the sample.

2.4.6. ESTIMATION OF UREA IN SERUM

Diacetyl Monoxime (DAM) Method (Martinek, 1969)

Procedure

All the reagents were brought to the room temperature before using the test. The undiluted serum sample was used in this method. 3 sets of test tubes were taken and marked as the Blank, Standard and Test. 0.01 mL serum sample was taken in the test tube and then 0.01 mL of glucose standard reagent was added in standard test tube and 0.01 mL of distilled water in blank. 1.0 mL of urea reagent, acid reagent and DAM reagent was added to all the test tubes.

2.4.7. ESTIMATION OF CREATININE IN SERUM

(Alkaline picrate method) **(Bones *et al.*, 1945).**

Procedure

Deproteinization of sample

2.0 mL of picric acid reagent was added in 0.2 mL of the serum sample. It was mixed well and centrifuged at 2500 – 3000 rpm for 10 minutes to obtain a clear supernatant. The supernatant obtained was used for the estimation.

All the reagents were brought to the room temperature before the test. The supernatant was used in this method. Three set of test tubes and marked as the Blank, Standard and Test. 1.1 mL of supernatant was taken in the test tube, then 0.1mL of creatinine standard was added in a standard test tube. Finally 0.1 mL of distilled water was added to the Blank test tube. 1.0 mL of picric acid reagent was added in the blank and standard test tubes alone. 0.1 mL of buffer reagent was added to all the test tubes. These solutions were mixed well and kept at room temperature for 20 minutes. The absorbance was read at 520 nm against a reagent blank.

Normal reference value**:** Serum creatinine: 0.5-1.1 mg/dL.

ESTIMATION OF CHOLESTEROL
(Wybenga and Pileggi et., al 1970)
Principle
Cholesterol reacts with hot solution of ferric perchlorate, Ethyl aceate and sulphuric acid (Cholesterol reagent) and given a lavender coloured complex with is measured at 560nm.

HAEMATOLOGICAL PARAMETERS
BLOOD SMEAR AND STAINING METHODS
The slide used to make the smear is known as the spreader. The spreader is a smooth edged glass slide.

SMEAR PREPARATION
A drop of blood was placed on one end of the glass slide. The spreader was brought to contact the drop of blood and then pushed forward.

The thin part is the tail part of the smear. The thickest part is the hard part of the smear. The middle part of the smear is known as the body of the smear.

Thin smear is done for a differential count of WBC and blood pictures.

STAINING
After smearing, making, staining was carried out. The labeled blood film has to be stained to differentiate the various blood cells.

TOTAL RED BLOOD CELLS COUNT- DIRECT METHOD
TEST PROCEDURE
Rat's blood was withdrawn up to 0.5 marking in the RBC pipette and simultaneously aspirated RBC diluting fluid up to 101 marking. Mixed well, waited for 5 minutes. This gave 1/200 dilution.

After discarding the stem fluid, charged with the mixer in the chamber. It was covered, with a cover glass. By holding, the tip of the RBC pipette at an angle of 45^{o}C, the chamber was charged. RBC's were counted at the counter heavy ruled squared area of four outer corners and one center square.

TOTAL LEUKOCYTE COUNT-DIRECT METHOD
TEST PROCEDURE
Rat's blood was withdrawn up to 0.5 marks in the WBC pipette and simultaneously aspirated WBC diluting fluid up to 11 marking, mixed well and waited for 5 minutes. This gave 1/20 dilution.

After discarding the stem fluid charge the chamber which has been covered with a cover glass. The tip of the WBC pipette was hold at a 45^{o} angle. After waiting for five minutes, the cells were counted the WBC's at 4 corners of the squares.

Statistical analysis
Results were presented as means_ SD. Data were analyzed by using one-way ANOVA and Student's t-test. The p value less than 0.05was regarded as statistically significant.

RESULTS
Clinical observation
During the period of Aflatoxin exposure the rats were observed for the behavioral changes. All the experimental group of animals appeared normal during the first week of toxin administration. They exhibited reduced feed intake and less active during the pronged period of toxin exposure. Feed refusal and reduction in body weight was pronounced in test groups (table 1) which were administered with higher concentrations of Aflatoxin. No abnormality was observed in control group of rats.

BLOOD BIOCHEMISTRY
During the period of aflatoxin exposure blood samples were collected from the experimental group of animals at the interval of 7 days up to 7 weeks to investigate the effect of Aflatoxin B1 on various biochemical and haematological parameters. Similarly, blood sample collections were made for analysis from the control group of animals. The results of the blood biochemistry showed that the Aflatoxin has a telling effect on the vital organs of the body like liver, kidney and immunological functions in the aflatoxin B1 exposed animals.

Both the SGOT and SGPT activity (table 2 &3) were found to be increased in the experimental rats that were exposed to aflatoxin B1. The increased level of SGOT and SGPT activity were observed with the increase in the dosage level of aflatoxin B1 and period of exposure.

Total protein
The total protein content was significantly decreased in the blood plasma after treatment with aflatoxin B1 for a period of 49 days. The decreased concentration of protein was observed in rats treated with highest dosage level of aflatoxin B1 than the other dosage limits. The decreased percentage of protein was noted to be 40% compared to the control groups (table 4).

Urea and Creatinine
Urea (table 5) and creatinine (table 6) content were increased significantly in the blood samples of aflatoxin mix exposed rats when compared to control.

Total cholesterol
The results of total cholesterol in the test groups showed decreased values comparing to the control one. The obvious changes of these parameters implied the impairment of liver function. The decreased values of these two parameters correspond to the increasing concentration of toxin and exposure period (table 7).

Alkaline phosphatase activity

During the initial period of exposure (7, 21, 28th day) to aflatoxin B1 the alkaline phosphatase activity was close to normal. A significant increase in the alkaline phosphatase activity was noted in test groups that were fed with highest concentration of aflatoxin B1 (17mg/kg body weight) at the 49th day of exposure period and this shows that there appeared a trend of dose-dependent increases in the activity of alkaline phosphatase (table 8). Increase in the alkaline phosphatase activity indicates liver damage in the rats exposed to the toxicant.

HEMATOLOGICAL PARAMETERS

Haematological parameters of experimental group of rats exposed to Aflatoxin B1 for 49 days (table 9) showed marked and significant decrease in erythrocytes (RBC) as the concentration of toxin increased when compared to control values. The results of the leukocyte count are summarized in table 10. Total leukocyte count has markedly decreased in the test group of animals when compared to the control groups. The decrease in the WBC count was phenomenal and proportional with the increased dosage of the aflatoxin and the period of exposure.

Table 1.Body weight of control and experimental group of rats during experimentation

Aflatoxin concentration mg/kg BW	Days of exposure period													
	0		7		21		28		35		42		49	
	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.
5	121±0.25	120±1.0	126±1.55	129±0.25	131±1.23	121±1.20	133±1.23	114±22	136±0.80	113±0.41	134±0.23	110±0.23	134±0.03	102±0.93
8	120±0.63	123±0.23	120±1.25	123±0.23	122±0.89	123±1.11	126±1.0	116±0.26	134±0.20	114±0.62	132±0.23	104±0.27	132±0.53	106±0.03
11	118±0.56	125±0.80	124±0.42	124±0.41	126±1.87	115±1.00	130±1.08	104±0.29	133±0.09	103±0.21	140±0.20	99±0.01	141±0.23	94±0.01
14	122±0.45	123±0.24	123±1.20	120±0.20	126±1.81	113±0.20	131±1.04	109±0.45	132±0.03	97±0.23	137±0.11	93±0.05	136±0.04	86±0.43
17	120±1.78	124±1.47	126±1.78	125±1.56	130±1.43	111±1.25	134±1.25	98±1.29	135±0.98	95±1.32	136±1.33	86±1.75	138±1.25	75±1.20

Experiments were performed three replicates ± Standard deviation. Statistical significance P<0.05
con – control
exp – experiment

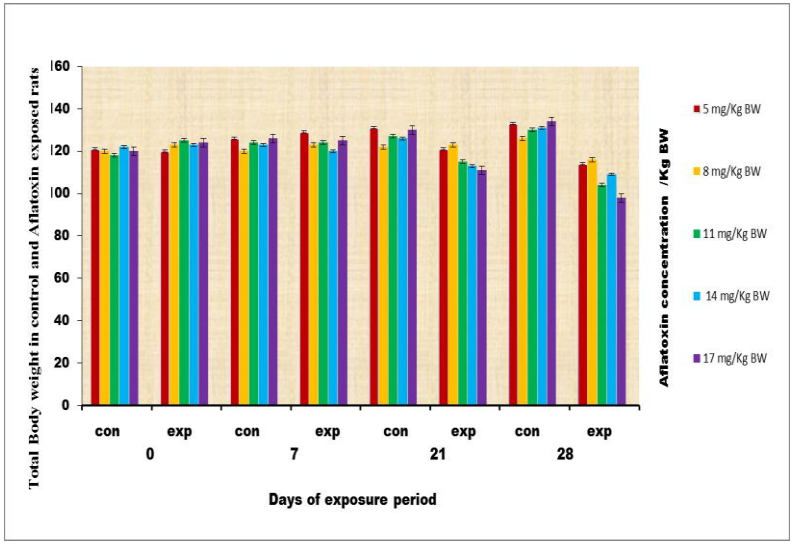


Fig 1. Body weight of control and experimental group of rats during experimentation

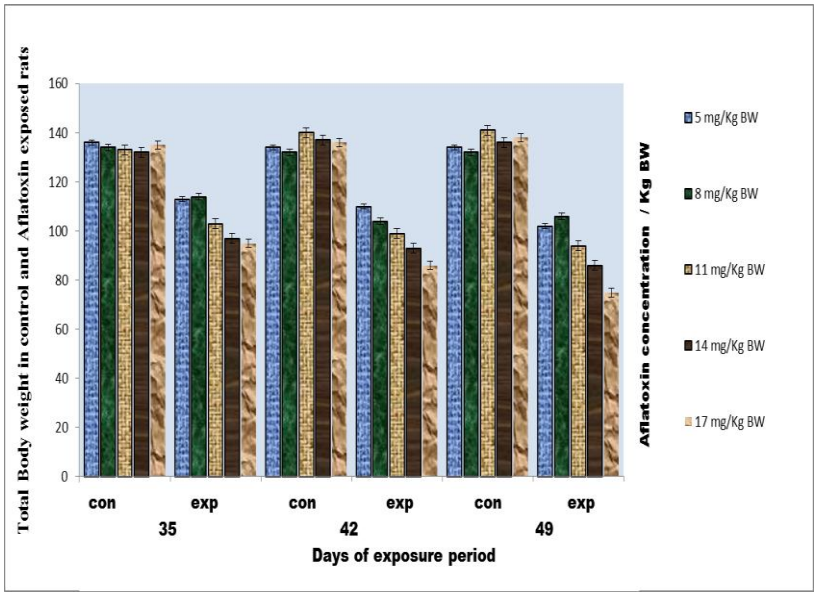


Fig 2 - Body weight of control and experimental group of rats during experimentation

Table 2. SGOT level in control and Aflatoxin exposed rats

Aflatoxin concentration mg/kg BW	Days of exposure period													
	0		7		21		28		35		42		49	
	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.
5	28±0.52	39±0.23	22±1.29	41±1.20	29±2.30	68±1.78	31±1.25	97±1.72	30±1.09	108±1.00	22±1.30	110±1.20	30±1.25	118±1.25
8	27±0.26	36±0.46	29±1.22	47±1.82	22±2.20	90±1.45	20±1.20	112±1.00	19±1.03	120±1.20	20±1.21	122±1.23	25±1.26	122±1.29
11	19±0.47	42±0.13	21±1.11	77±1.54	23±2.26	117±1.03	19±1.20	120±1.04	17±1.60	122±1.93	20±1.72	123±1.00	22±1.50	126±1.00
14	22±1.24	39±1.20	25±1.10	89±2.26	24±0.23	119±2.20	31±1.46	122±1.16	33±1.70	127±1.20	27±2.20	130±2.63	23±1.90	132±1.11
17	18±2.33	40±2.32	22±1.98	107±2.56	20±1.89	127±2.12	17±2.63	130±2..23	23±3.43	133±2.88	28±2.76	133±3..14	24±2.56	134±3.24

Experiments were performed three replicates ± Standard deviation. Statistical significance P<0.05.

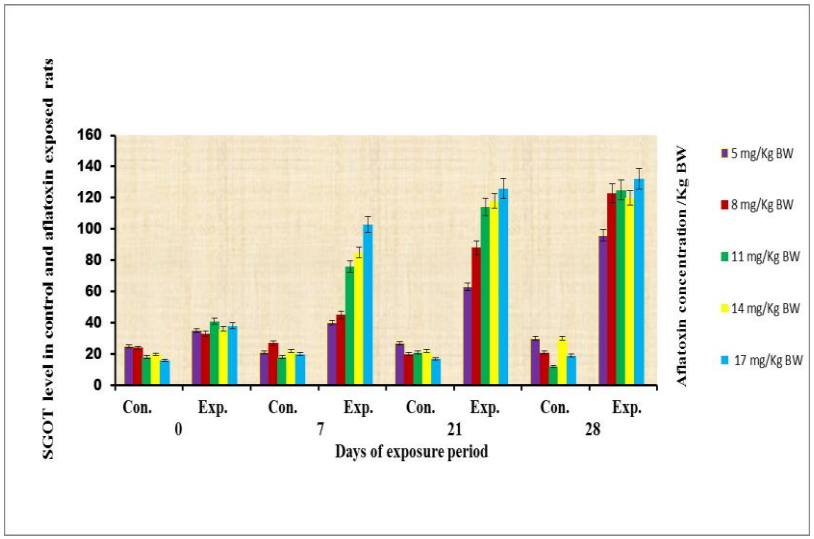


Fig 3 -SGOT level in control and Aflatoxin exposed rats

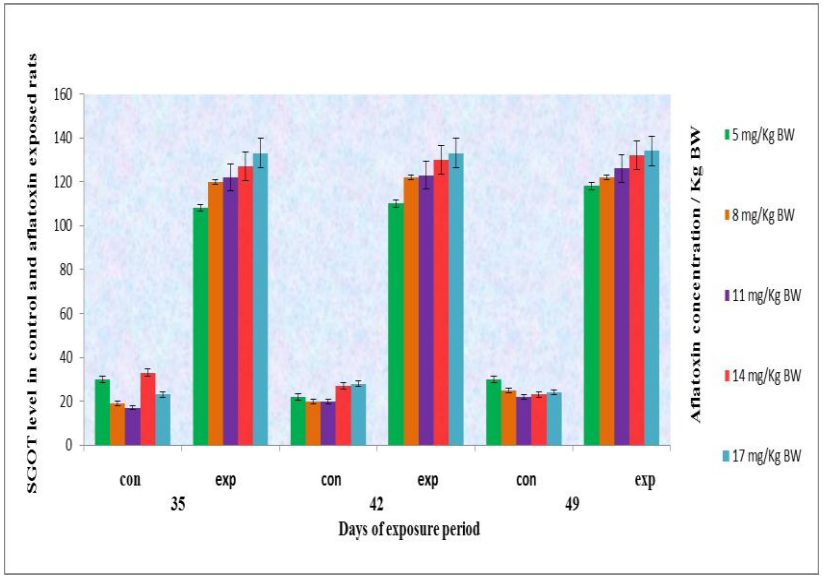


Fig 4 - SGOT level in control and Aflatoxin exposed rats

Table 3. SGPT level in control and Aflatoxin exposed rats

Aflatoxin concentration mg/kg BW	Days of exposure period													
	0		7		21		28		35		42		49	
	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.
5	20.3± 0.58	24.2±	21.7± 1.05	22.4± 0.95	24.6± 0.54	26.1± 0.29	27.7± 0.45	23.4± 2.01	21± 2.01	22.1± 2.17	21± 2.09	25± 1.47	28.8± 0.78	29.8± 0.89
8	15.3± 0.89	19.4± 1.56	19.9± 1.02	21.1± 0.61	16.9± 0.84	21.7± 0.47	15.1± 0.42	27± 1.23	28.7± 2.78	27.8± 0.85	28± 3.45	28.9± 1.11	24.1± 1.97	24.8± 1.24
11	22.1± 0.41	25.4± 1.63	25.9± 0.45	23.1± 1.02	26.8± 1.11	25± 1.14	23.9± 1.05	26.8± 1.27	27± 2.98	27± 1.28	27.4± 0.52	23± 1.47	32.5± 1.52	22.1± 1.26
14	23.2± 0.25	21.2± 1.23	24.3± 0.56	20.5± 0.84	22.4± 1.89	24.4± 1.05	34.5± 0.63	22.8± 1.45	33.3± 3.00	23.5±	37.6± 1.89	25± 1.02	34.1± 1.67	23.2± 0.74
17	19.7± 0.48	18.6± 1.59	28.5± 0.95	21.1± 1.78	29.7± 1.89	18.4± 0.73	30.6± 3.00	27± 2.05	36.7± 1.28	25.9±	38.2± 1.78	24± 2.56	42.6± 1.78	19.7± 2.40

Experiments were performed three replicates ± Standard deviation. Statistical significance P<0.05.

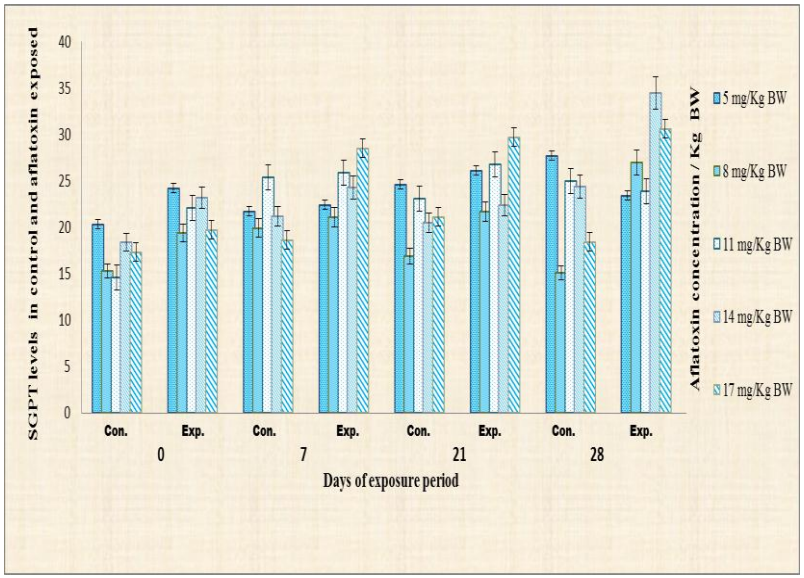


Fig 5- SGPT level in control and Aflatoxin exposed rats

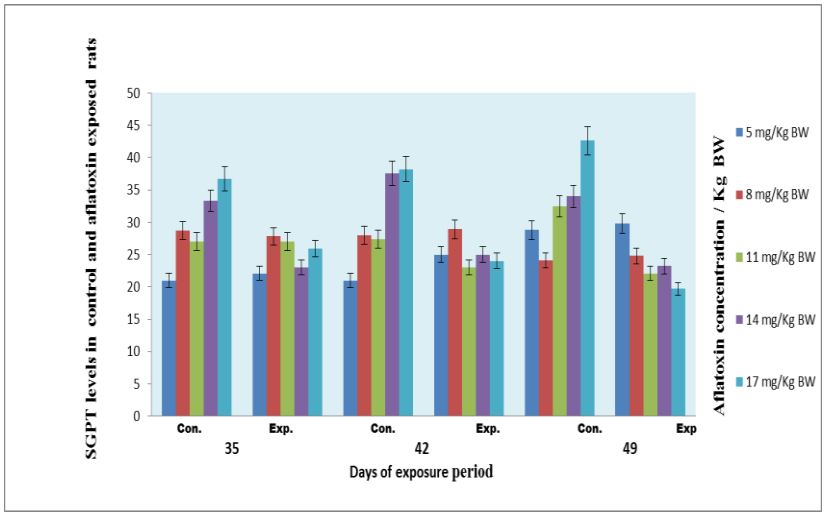


Fig – 6 SGPT level in control and Aflatoxin exposed rats

Table 4. Total protein level in control and Aflatoxin exposed rats

Aflatoxin concentration mg/kg BW	Days of exposure period													
	0		7		21		28		35		42		49	
	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.
20	6.5±1.02	6.9±2.04	6.4±0.78	7.2±2.04	7.3±0.67	7.2±0.65	6.2±0.14	7.3±0.64	6.3±1.06	6.4±1.04	6.6±2.04	6.9±1.65	7.2±0.63	7.1±0.58
25	7.8±1.69	7.1±2.08	6.6±0.79	7.6±0.98	7.3±0.87	7.6±0.21	6.4±0.65	6.6±1.27	6.7±1.21	6.3±1.34	7.0±2.05	6.6±1.62	7.4±0.22	6.8±0.98
30	7.1±1.45	7.2±1.00	6.4±0.72	7.6±0.68	7.4±0.64	6.4±0.65	7.3±0.41	6.4±0.98	6.3±1.23	6±1.00	7.5±1.74	5.4±1.36	7.8±1.26	5.4±1.00
35	7.3±1.14	6.7±1.06	7.3±0.64	6.1±0.24	6.6±0.54	6.1±0.36	7.6±0.36	6±1.81	7.6±1.65	5.7±1.05	7.3±1.56	5.3±1.32	6.7±1.24	4.3±1.02
40	7.2±1.01	7±3.00	7.5±2.04	6±0.26	6.3±0.28	5±0.27	8±0.58	5.6±1.26	7.8±1.61	5.1±3.01	7.4±1.05	4.9±0.36	7.7±1.35	4.7±1.06

Experiments were performed three replicates ± Standard deviation. Statistical significance P<0.05.

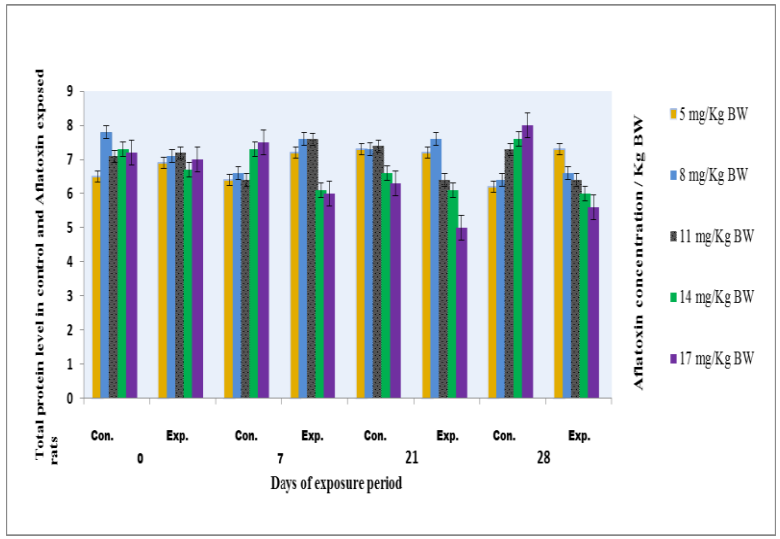


Fig 7 - Total protein level in control and Aflatoxin exposed rats

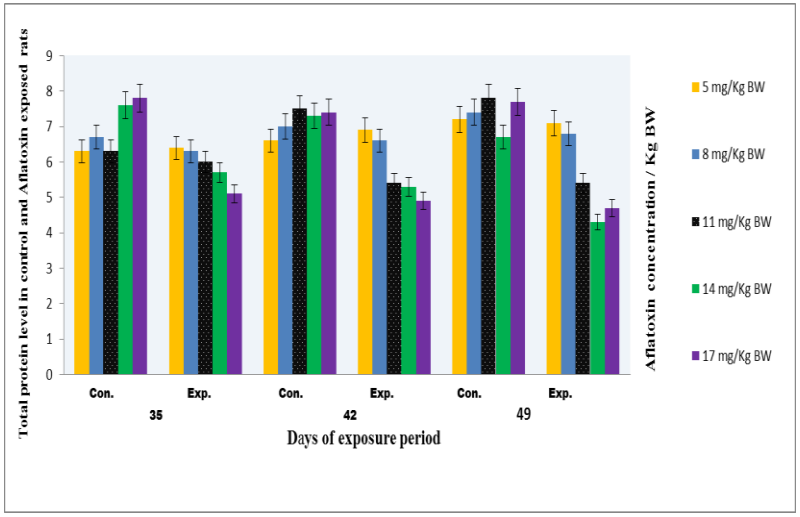


Fig 8 -Total protein level in control and Aflatoxin exposed rats

Table 5- Urea level in control and Aflatoxin exposed rats

Aflatoxin concentration Mg/Kg BW	Days of exposure period													
	0		7		21		28		35		42		49	
	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.
5	26±1.08	26±0.65	26±0.87	24±0.41	30±1.00	37±0.37	28±0.27	39±0.64	24±0.47	49±0.61	23±1.07	53±0.83	31±0.11	50±0.89
8	26±0.85	24±0.98	32±1.07	36±0.23	25±2.07	34±1.24	32±0..91	34±0.52	33±0.26	47±0.46	35±2.74	59±2.04	27±1.07	56±0.24
11	29±1.56	32±0.46	35±2.50	35±0.11	28±0.16	38±1.86	25±0.31	42±0.62	31±1.075	50±0.74	31±1.43	61±2.06	30±1.78	65±0.62
14	27±1.12	20±0.97	30±1.22	32±0.91	33±1.14	51±0.74	31±0.19	56±0.67	27±1.27	66±0.79	29±091	72±2.04	31±1.00	72±0.71
17	25±0.78	23±0.45	28±	34±0.71	30±3.00	52±1.17	32±0.47	61±0.66	29±1.00	69±1.08	30±0.78	74±1.08	27±0.22	76±3.02

Experiments were performed three replicates ± Standard deviation. Statistical significance P<0.05.

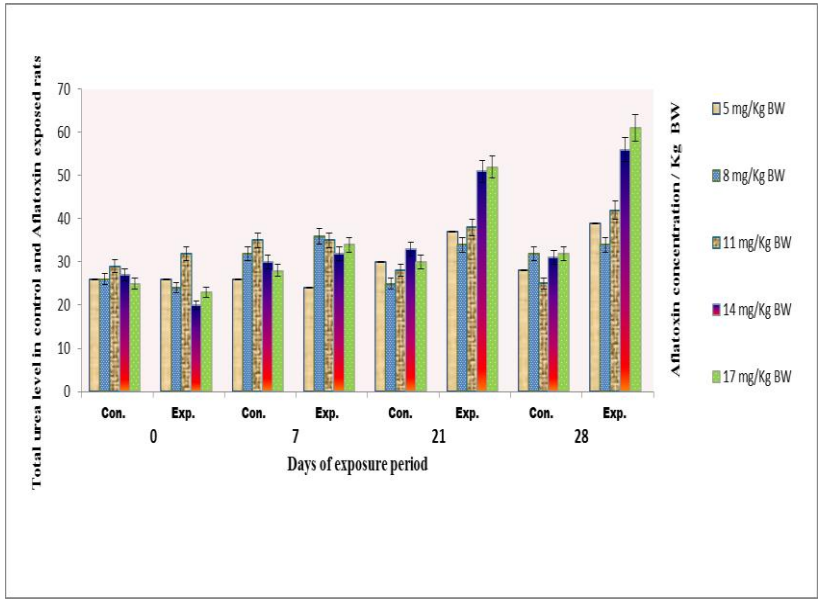


Fig 9- Urea level in control and Aflatoxin exposed rats

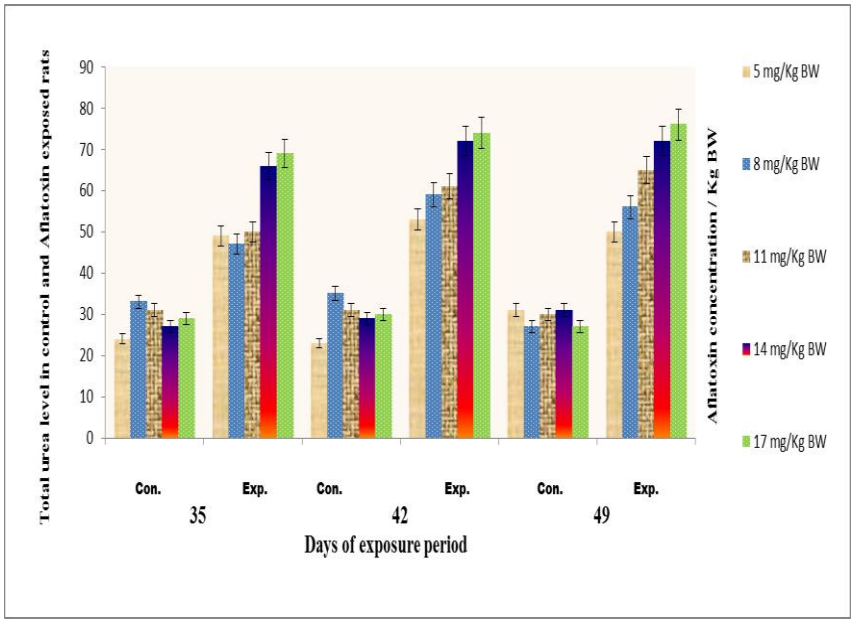


Fig 10 - Urea level in control and Aflatoxin exposed rats

Table 6- Creatinine content in control and Aflatoxin exposed rats

Aflatoxin concentration mg/kg BW	Days of exposure period													
	0		7		21		28		35		42		49	
	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.
5	0.8±0.05	0.6±0.02	0.9±0.04	1.2±2.00	1.0±0.03	1.0±0.04	0.6±0.02	0.7±0.89	0.8±0.49	1.0±0.66	1.0±0.02	1.3±0.01	1.3±0.46	1.2±0.08
8	0.7±0.02	0.8±0.04	0.8±0.09	0.9±0..25	0.7±1.025	0.8±0.07	0.8±0.03	0.9±0.06	0.7±0.66	1.4±0.16	0.9±0.08	1.4±0.05	0.9±0.64	1.8±0.14
11	0.6±0.01	0.9±0.32	0.8±0.07	0.9±0.02	0.8±0.01	0.9±0.08	0.9±0.81	1.0±0.04	0.8±0.81	1.4±0.81	0.8±0.02	1.90.62	0.8±0.63	2.4±0.07
14	0.9±0.01	0.8±0.21	0.7±0.91	0.6±0.12	0.9±0.78	1.3±0.05	0.9±0.11	1.9±0.14	0.9±0.07	1.9±0.01	1.1±0.03	2.5±0.22	0.9±0.16	4.6±0.03
17	0.7±0.02	0.7±0.01	0.9±0.04	1.7±0.11	0.8±0.016	1.9±0.19	1.0±0.41	2.0±0.16	1.1±0.27	3.4±0.05	1.0±0.03	5.2±0.68	1.0±0.05	9.6±0.28

Experiments were performed three replicates ± Standard deviation. Statistical significance P<0.05.

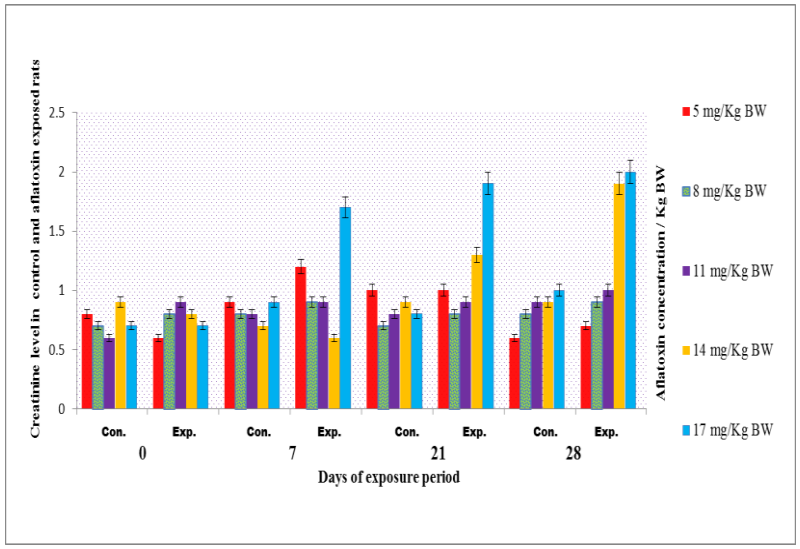


Fig 11 -Creatinine content in control and Aflatoxin exposed rats

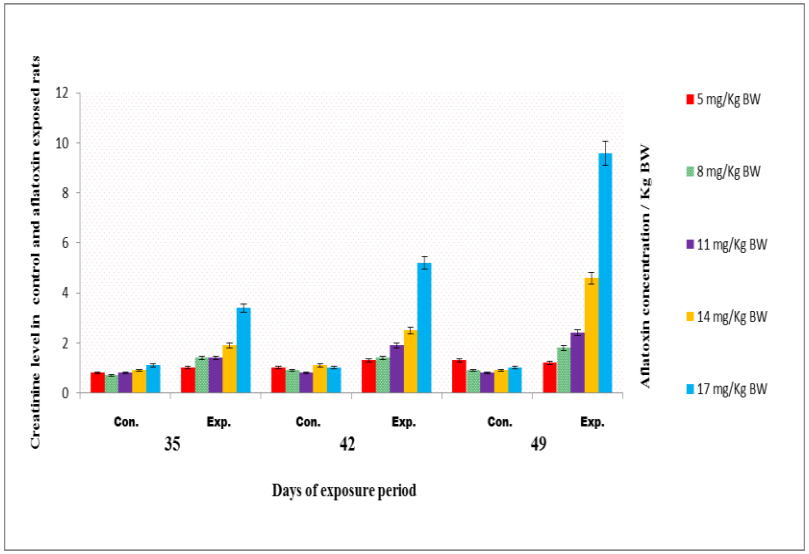


Fig 12 - Creatinine content in control and Aflatoxin exposed rats

Table 7- Total cholesterol content in control and Aflatoxin exposed rats

Aflatoxin concentration mg/kg BW	Days of exposure period													
	0		7		21		28		35		42		49	
	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.
5	154±0.54	165±0.88	153±1.65	178±0.64	175±1.63	164±1.62	176±1.08	170±1.63	166±0.48	173±0.91	184±1.54	192±1.58	177±1.56	185±1.5
8	152±0.46	172±0.95	164±1.82	174±0.96	168±2.29	1851.86	174±1.63	174±0.93	174±1.02	184±1.52	169±10.5	163±1.65	196±0.24	177±1.53
11	164±0.95	187±0.63	183±1.62	186±1.03	193±1.63	182±1.06	185±1.25	138±0.53	166±2.16	131±1.83	171±1.14	133±.025	180±1.63	130±1.04
14	166±0.78	175±0.46	175±1.84	121±3.02	165±1.35	125±1.62	186±1.63	116±1.4	171±1.63	125±3.20	187±1.04	116±1.05	164±0.64	108±1.05
17	173±1.56	174±1.25	183±1.96	119±2.65	162±0.98	99±1.82	199±1.05	91±1.86	187±2.08	89±1.02	163±1.20	82±1.00	172±1.58	86±3.00

Experiments were performed three replicates ± Standard deviation. Statistical significance P<0.05.

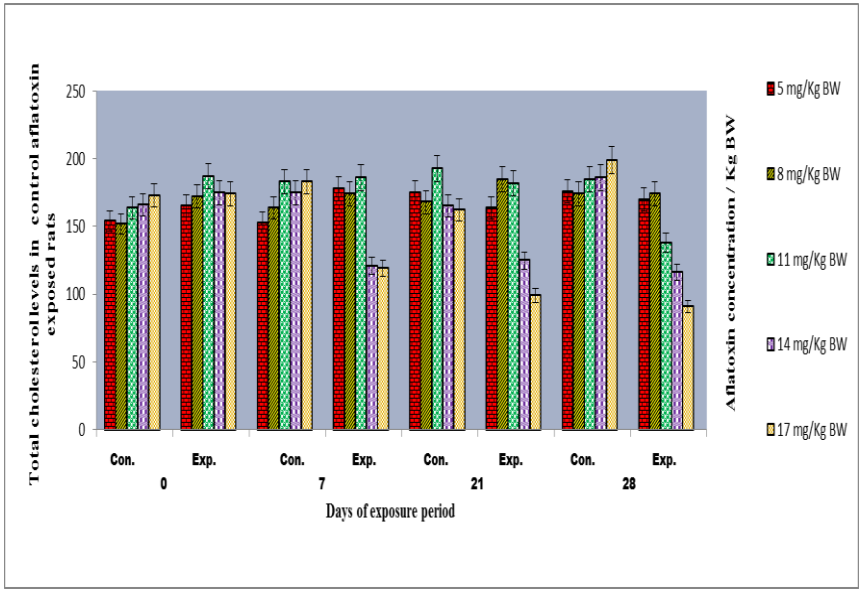


Fig 13 - Total cholesterol content in control and Aflatoxin exposed rats

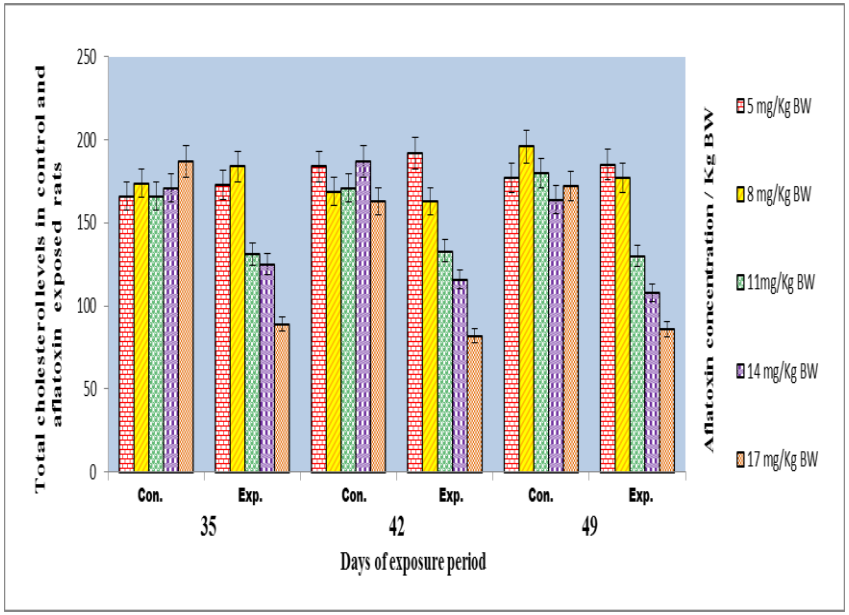


Fig 14- Total cholesterol content in control and Aflatoxin exposed rats

Table 8- Alkaline phosphatase activity in control and Aflatoxin exposed rats

flatoxin concentration mg/kg BW	Days of exposure period													
	0		7		21		28		35		42		49	
	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.
5	112±1.26	114±1.90	116±1.25	124±2.03	124±1.23	134±1.54	106±1.48	168±1.45	112±0.90	186±1.66	128±0.90	220±0.90	117±0.90	284±1.85
8	121±1.95	121±1.55	122±1.08	127±1.87	121±1.65	149±1.68	112±1.14	162±0.90	118±1.08	196±1.97	128±1.97	244±0.90	129±1.89	311±2.90
11	112±0.90	102±1.89	127±1.26	167±1.01	114±1.54	211±1.98	124±1.87	121±0.91	274±1.97	126±1.87	313±1.11	122±2.89	363±2.30	136±2.00
14	123±1.78	112±1.02	108±1.90	198±1.17	125±1.06	263±2.08	305±2.00	121±0.89	364±0.98	132±1.29	373±1.08	132±2.04	375±3.02	305±2.54
17	98±2.00	107±0.87	113±1.35	226±2.14	121±1.02	264±1.79	124±2.04	350±1.79	128±1.87	380±1.67	106±1.64	392±1.08	125±2.58	394±2.65

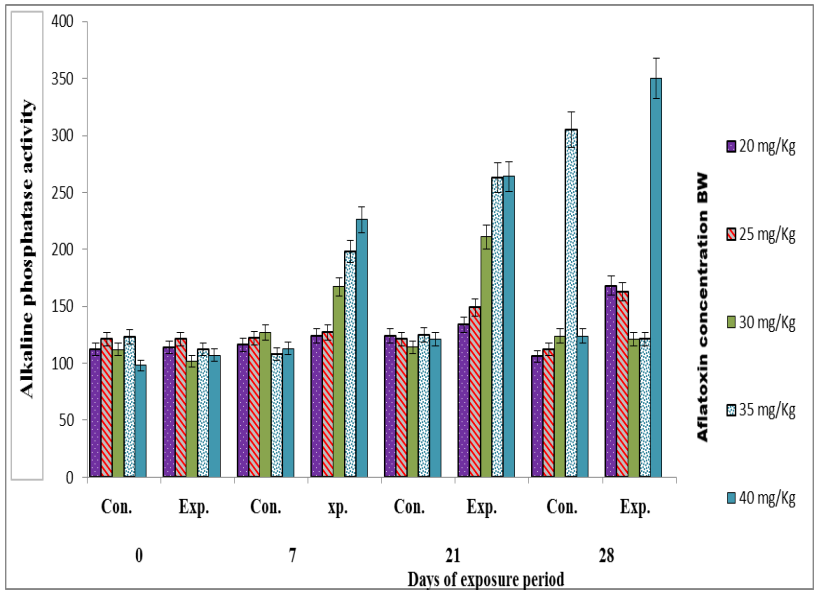


Fig 15 - Alkaline phosphatase activity in control and Aflatoxin exposed rats 0-28 Days of exposure period

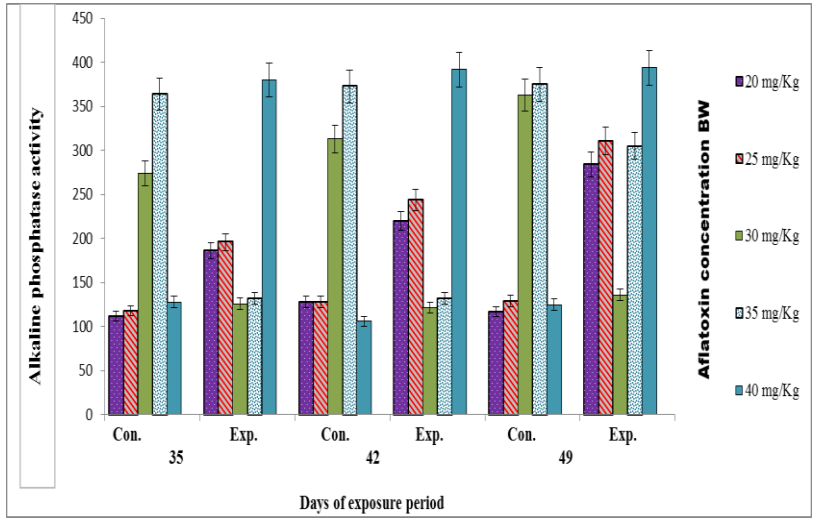
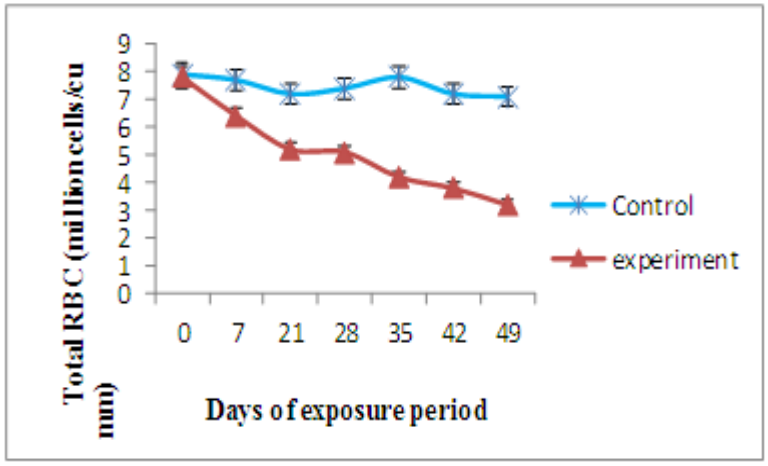


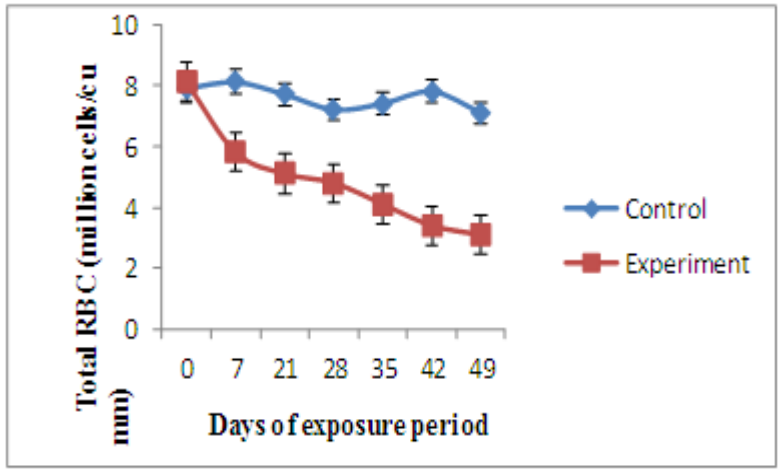
Fig 16 - Alkaline phosphatase activity in control and Aflatoxin exposed rats 35-49 Days of exposure period

Table 9- in Total RBC (million cells/cu mm) count control and Aflatoxin exposed rats

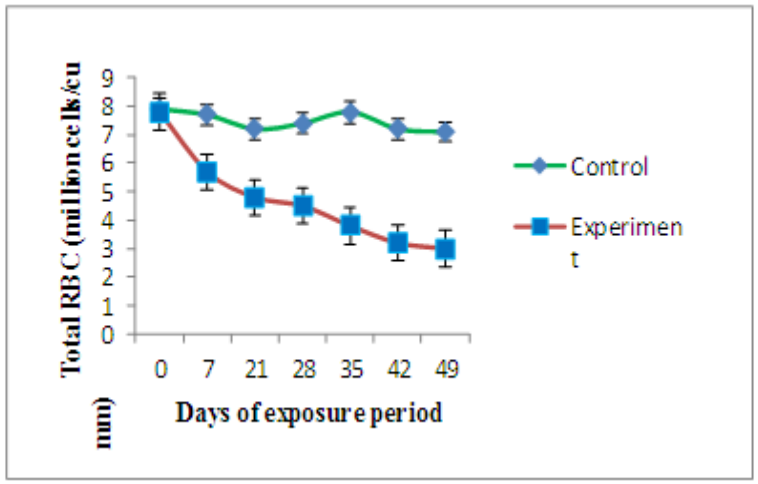
Aflatoxin concentration mg/kg BW	Days of exposure period													
	0		7		21		28		35		42		49	
	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.
5	7.90±0.98	7.80±0.87	7.70±0.67	6.40±0.64	7.20±1.08	5.20±0.87	7.40±1.02	5.10±1.24	7.80±1.56	4.20±1.04	7.20±1.66	3.80±1.56	7.10±1.55	3.20±2.04
8	7.90±1.00	7.70±0.98	7.70±1.64	6.20±0.54	7.10±1.64	5.10±0.37	7.40±1.00	5.10±1.87	7.80±0.58	4.10±0.57	7.10±1.08	3.40±1.64	7.10±1.07	3.10±1.014
11	7.90±1.00	7.90±1.08	7.70±1.57	5.80±0.98	7.20±1.87	5.10±0.97	7.20±1.02	4.80±1.20	7.80±0.64	4.10±0.67	7.20±1.06	3.40±1.02	7.10±0.74	3.10±1.07
14	7.90±1.04	7.80±1.07	7.70±1.61	5.70±1.08	7.20±1.08	4.80±0.94	7.30±1.04	4.50±1.64	7.80±1.54	3.80±2.00	7.10±1.04	3.20±1.08	7.10±1.11	3.00±1.64
17	7.90±1.01	7.90±1.45	7.70±1.08	5.70±1.058	7.20±1.07	4.20±1.03	7.40±1.32	4.20±0.94	7.80±1.324	3.80±1.24	7.20±1.04	3.20±1.04	7.10±0.09	3.00±2.00



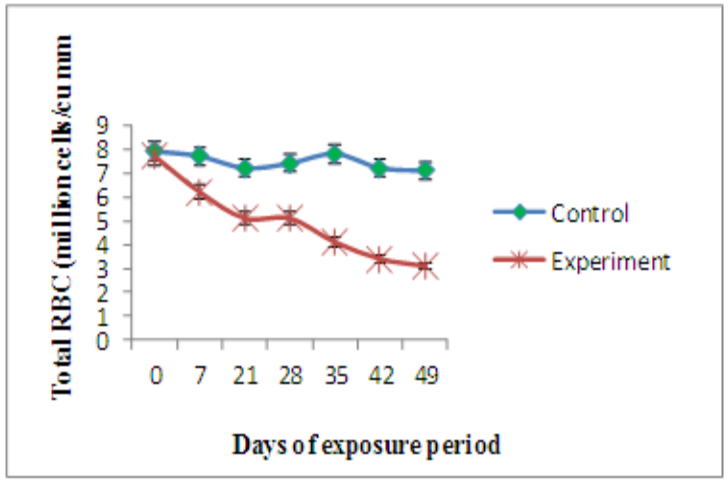
Total RBC count in control and Test with Aflatoxin conc.5 mg/kg BW



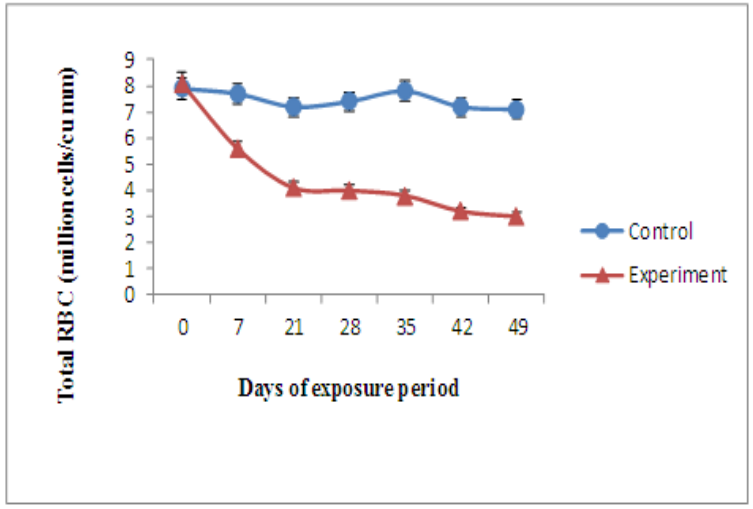
Total RBC count in control and Test with Aflatoxin conc.8 mg/kg BW



Total RBC count in control and Test with Aflatoxin conc.11 mg/kg



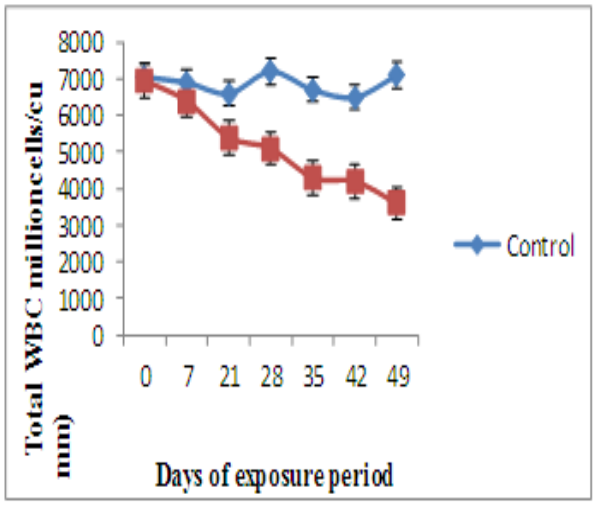
Total RBC count in control and Test with Aflatoxin conc.14mg/kg BW



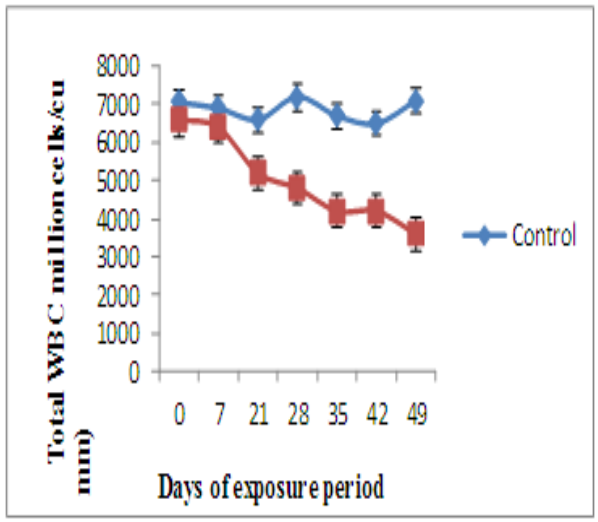
Total RBC count in control and Test with Aflatoxin conc.17 mg/kg BW
Fig 17-21 Total RBC count in different Aflatoxin concentration fed animals

Table 10 - Total WBC (cells/cu mm) count in control and Aflatoxin exposed rats

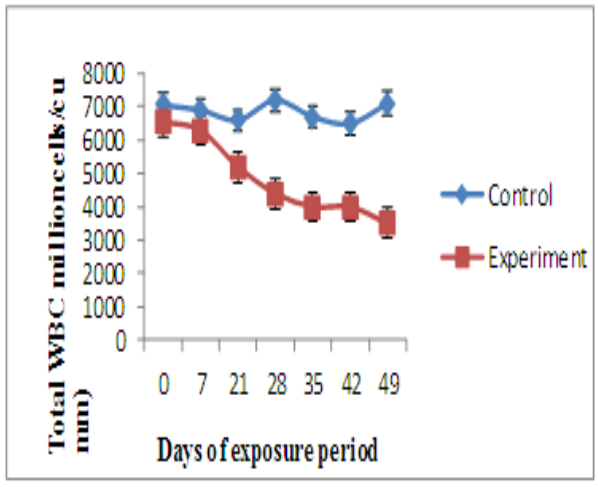
Aflatoxin concentration mg/kg BW	Days of exposure period													
	0		7		21		28		35		42		49	
	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.	con	exp.
5	7050± 0.98	6950± 0.70	6900± 0.65	6400± 0.95	6600± 0.75	5400± 0.95	7200± 1.95	5100± 0.47	6700± 0.95	4300± 1.95	6500± 0.97	4200± 0.29	7100± 1.95	3600± 0.95
8	7050± 1.24	6600± 0.24	6900± 1.02	6400± 1.27	6600± 1.97	5200± 1.06	7200± 2.60	4800± 0.64	6700± 2.03	4200± 1.67	6500± 2.04	4200± 1.14	7100± 1.47	3600± 1.87
11	7050± 1.20	6550± 0.94	6900± 1.25	6300± 1.07	6600± 2.06	5200± 2.04	7200± 1.09	4400± 0.89	6700± 1.45	4000± 0.34	6500± 1.36	4000± 1.36	7100± 1.04	3500± 1.64
14	7050± 1.08	6500± 1.02	6900± 0.95	6000± 1.86	6600± 0.69	5000± 1.00	7200± 1.04	4400± 1.02	6700± 1.06	4000± 1.00	6500± 1.24	3800± 0.97	7100± 1.00	2900± 0.99
17	7050± 1.69	6400± 2.00	6900± 1.20	5800± 1.65	6600± 2.06	4900± 1.00	7200± 1.02	4200± 1.71	6700± 1.35	4000± 3.04	6500± 1.09	3600± 0.36	7100± 0.94	2800± 2.04



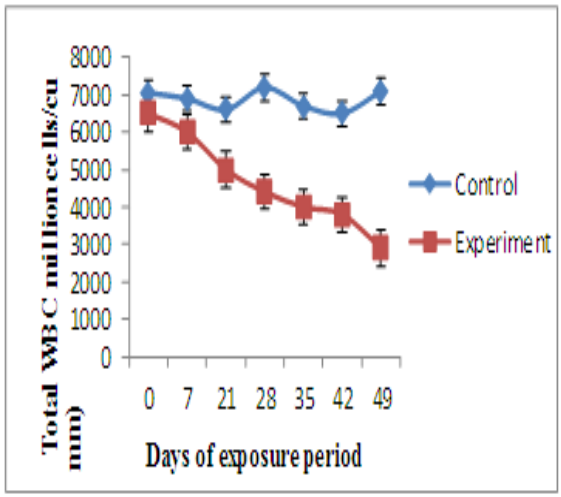
Total WBC count in control and Test with Aflatoxin conc.5 mg/kg BW



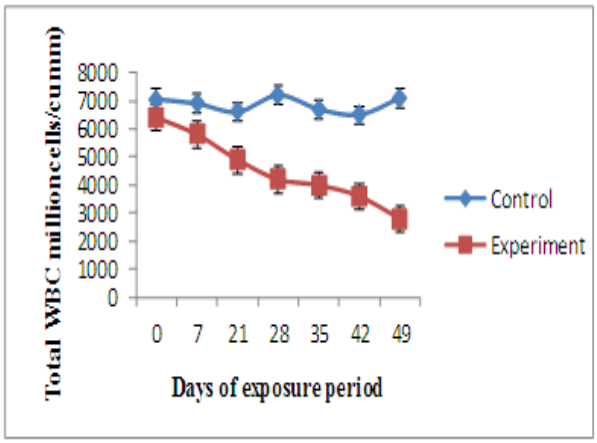
Total WBC count in control and Test with Aflatoxin conc.8 mg/kg BW



Total WBC count in control and Test with Aflatoxin conc.11 mg/kg BW



Total WBC count in control and Test with Aflatoxin conc.14 mg/kg BW



Total WBC count in control and Test with Aflatoxin conc.17mg/kg BW

Fig 22-26 Total WBC count in different Aflatoxin concentration fed animals

DISCUSSION

Humans, as well as animals, are exposed to mycotoxins in their diets, and this can be considered, in most cases, the route of administration of natural intoxications caused by these compounds. Similarly the test animals in the present study were infused with aflatoxin B1 along the feed material. The results of the various biochemical and hematological parameters in the aflatoxin exposed rats reveal possible damage to vital physiological organs of the animals like liver, kidney and immunological functions. The results have also revealed that the abnormalities in various parameters assessed correspond with the increase in the concentration of the toxicant and the period of exposure. Toxigenic molds growing on the food and feed materials contaminate them with all the toxicants (AFB_1, AFB_2, AFG_1 and AFG2). Of all the aflatoxin, aflatoxin B1 (AFB1) was reported to be the most toxic and classified as group 1 carcinogen. Hence it was chosen to study the toxic effect of aflatoxin B1 in the present investigation. Various blood biochemical

parameters involve in several biological pathways and immune mechanisms are critical factors when considering the biochemical and physiological effects of aflatoxin.

Biochemical parameters are the best indicators of stress situations caused by intoxication with pesticides or chemicals. The results of the various biochemical parameters of the experimental rats exposed to aflatoxin B1 clearly indicate the stress situation and possible damage to the vital organs of the test animals. The liver being the center for detoxification of foreign compound that enters the body made it to be exposed to environmental toxins and chemicals present in food or drink. Many of the biochemical parameters assessed have deviated from their normal values. Alkaline phosphatase activity was increased in the experimental groups that were fed with higher concentration of aflatoxin B1 (14 and 17 mg /kg BW table 12). Whereas the activity of alkaline phosphatase was close to normal and not significantly altered in the experimental rats that were fed with lower levels of aflatoxin B1 (5, 8 and 11mg/kg BW) when compared with the control group. The increased activity of alkaline phosphatase in the present study might have been induced due to the intoxication of experimental rats with aflatoxin mix. Similar results have been reported by Theumer et al., 2002 in rat serum that were exposed to subchronic levels of *Fusarium* mycotoxin (FB_1). The intoxication of aflatoxin B1 produced an increase of serum ALP activity in these animals, possibly caused by the severe toxic action of aflatoxin on the liver cells. A change in blood chemistry implies the occurrence of liver injury, and remarkable hepatic oxidative damage. Liu et al., 1999 have observed butenolide induced changes in blood chemistry and remarkable hepatic oxidative damage in experimental rats. Moreover liver contain many enzymes and proteins possible leakage of these enzymes across damaged liver cells could have led to the elevated levels of ALP in the test group of animals. Toxin chelating may disrupt the liver tissue by disintegrating the functional and structural properties of the cells. Liver being an organ for inter conversion and storage of food stuff and a center of all oxidative and detoxification mechanisms show maximum alteration in its tissue composition. Liver plays an important role because all substances absorbed by the gastrointestinal tract pass through it before entering in to the general circulation. Some toxicant cause direct injury to the liver other converts the chemicals into toxic substances through metabolic conversion.

Blood contains a host of proteins especially functional types mediating inflammatory reactions, blood coagulation, and transport and receptor proteins. The declined total protein in the blood plasma could be due to energy demand required for the removal of aflatoxin and its metabolic waste products from the animal body. Such decline in the protein content in several body tissues of animals and humans exposed to various chemical and pesticide intoxications have been reported(Das et al.,2003, Yeragi et al., 2003 Bedii and Kenan, 2005).

The direct effect of aflatoxin (AFB_1) on the serine protease, an important enzyme that mediates cascade mechanism on blood coagulation has been reported by Hsieh et al., 1986. Aflatoxin infused in to the animals may bind with the functional protein causing the loss of protein activity, particularly in the case of enzymes. Aflatoxin intoxication (AFB1) poses another threat to the organism by promoting oxidative damage in proteins (Amici et al., 2007; Peng et al., 2007; Ubagai et al., 2008). Aflatoxin also inhibits some serine proteolytic enzymes (Clausen et al., 2002) responsible for the degradation of damaged proteins, with consequent relevant implications in hepatocarcinogenesis (Peng et al., 2007). Ochratoxin-A (OTA) is a metabolite produced by *Aspergillus ochraceus* is known to modify the brain levels of free amino acids up on acute administration of the toxin (Belmadani A et al., 1998). Acute administration of OTA has recently been reported to cause decrease in the protein content and tyrosine hydroxylase activity leading to oxidative stress and DNA damage in all brain regions (Sava et al., 2006).

The effects of sub-chronic and chronic intake of AFB_1 on the immune system functionality in experimental animals have been reported by several authors. It has been observed that the AFB_1 ingestion has a toxic action on the immunologic system, being the cell-mediated immunity and the phagocytic cell functions the main affected compounds (Quist et al., 2000). Rotter et al. (1994) observed an increase in segmented neutrophils and total leukocytes, in pigs fed diets containing deoxynivalenol (DON). Prolonged exposure to aflatoxin causes acute aflatoxicosis as an inflammatory response and is related to neutrophil disruption and neutrophil elastase inhibition thereby affecting the phagocytosis and defense against infection by invading microorganisms (Wiedow and Meyer-Hoffert, 2005). Bharti Odhav et al., (2008) have reported that mycotoxins FB1and OTA, produced damage to the cell structure and intracellular organelles of lymphocytes and neutrophils in a dose dependent manner. Leukocyte death was evident in the decrease in cell survival when exposed to the mycotoxins. Leukocyte cell survival rate has decreaed with increasing toxin concentrations upon exposure to fusarium mycotoxins (Bharti Odhav et al., 2008). Raisuddin et al. (1993) informed dose dependent immune- suppression in weanling rats fed with AFB1 for four weeks.

Noticeable differences were observed in haematological parameters of aflatoxin B1 exposed rat after 35 days of exposure in the present study. The values of RBC and WBC showed a significant decrease with increase in concentration of aflatoxin B1 with the control group having the highest values and the group exposed to the highest concentration (17mg/Kg body weight) showed the lowest values. The rats fed with aflatoxin B1 (AFB1)

could have induced the decrease in total leukocytes and erythrocytes in the blood through some unknown mechanism. In *in vitro* study mycotoxins, FB1 and OTA, produce damage to the cell structure and intracellular organelles of lymphocytes and neutrophils in a dose dependent manner. Leukocyte death was evident when exposed to the mycotoxins and a decrease in cell survival was observed (Bharti Odhav et al., 2008). The decreased WBC counts in the experimental rats exposed to aflatoxin B1 clearly indicate the impairment of immune functions and eventually could lead to the susceptibility to chronic diseases and carcinogenisis. Changes in differential subset distributions and functional alterations of specific lymphocyte subsets have been correlated with aflatoxin exposure in Ghanaian adults and indicate that aflatoxins could cause impairment of human cellular immunity that could decrease resistance to infections (Jiang et al., 2005). The results of the present experiment show the immunosuppressive activity of aflatoxin and this could have important consequences in human and animal health as the breakdown in immunity may lead to the occurrence of disease even in properly vaccinated animals and humans.

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REFERENCES

1. Abd Alia, E.A.M., Neamat-Allah, A.A., Aly, S.E., Situation of mycotoxins in milk, dairy products and human milk in Egypt. Mycot. Res. 2000; 16: 91–100.
2. Adegoke, G.O., Letuma, P., Strategies for the Prevention and Reduction of ycotoxins in Developing Countries, in: Mycotoxin and Food Safety in Developing Countries. In Tech, 2013; 123: 136.
3. Adhikari, A., Sen, M.M., Gupta-Bhattacharya, S., Chanda, S., 2004. Airborne viable, non-viable, and allergenic fungi in a rural agricultural area of India: a 2-year study at five outdoor sampling stations. Science of the Total Environment, 326: 123-141.
4. Aly, S.A., Anwer, W., 2009. Effect of naturally contaminated feed with aflatoxins on performance of laying hens and the carryover of aflatoxin B residues in table eggs. Pakistan J. Nutr. 8(2): 181–186.
5. Amici, M., Cecarini, V., Pettinari, A., Bonfili, L., Angeletti, M., Barocci, S., Biagetti, M., Fioretti, E., Eleuteri, A.M., 2007. Binding of aflatoxins to the 20S proteasome: effects on enzyme functionality and implications for oxidative stress and apoptosis. Biol. Chem. 388: 107–117.
6. Anklam E., Stroka J, Boenke A, Food Control, 2002; 13: 173–183.
7. Averkieva, O., 2009. Mycotoxins in Grains Harvested in 2008: wheat. Kemin Industries, Inc. contamina – Windows Internet Explorer.
8. Bedii, CiCi. K and Kenan. Engin 2005. The effect of Cadmium on levels of glucose in serum and glycogen reserves in the liver and muscle tissue of *Cyprinus Carpio*.
9. Belmadani A, Tramu G, Betbeder AM, Steyn PS, Creppy EE. Regional selectivity to ochratoxin A, distribution and cytotoxicity in rat brain. Arch Toxicol. 1998; 72: 656–62.
10. Bergmeyer, H.U., Horder, M., Rej, R. 1986. International Federation of Clinical Chemistry IFCC methods for the measurement of catalytic concentrations of enzymes. J. Clin. Chem. Clin. Biochem., 24: 497.
11. Bharti Odhav, Jamila K. Adam, Kanti D. Bhoola., 2008. Modulating effects of fumonisin B1 and ochratoxin A on leukocytes and messenger cytokines of the human immune system. International Immunopharmacology, 2008; 8: 799–809.
12. Bones, R.W. Biochemistry in diagnosis. J. Biol. Chem. 1945; 158: 581.
13. Bryden, W.L., Mycotoxins in the food chain: human health implications. Asia Pac. J. Clin. Nutr. 2007; 16: 95–101.
14. Busby. W.F., Wogan. G.N., Aflatoxin, in: C.E. Searle (Ed.), Chemical Carcinogens, American Chemical Society, Washington, DC, 1984; 945–1136.
15. CAST, 2003. Mycotoxins: Risks in Plant, Animal and Human Systems. Report No. 139. Council for Agricultural Science and Technology, Ames, Iowa, USA.
16. Clark, H.A., Snedeker, S.M., Ochratoxin A: its cancer risk and potential for exposure. J. Toxicol. Environ. Health, Part B. Crit. Rev. 2006; 9: 265–296.
17. Clausen, T., Southan, C., Ehrmann, M., The HtrA family of proteases: implications for protein composition and cell fate. Mol. Cell. 2002; 10: 443–455.
18. Cleveland, T.E., Dowd, P.F., Desjardins, A.E., Bhatnagar, D., Cotty, P.J., United States Department of Agriculture Agricultural Research Service research on pre-harvest prevention of mycotoxins and mycotoxigenic fungi in US crops. Pest Manag. Sci. 2003; 59: 629 642. doi:10.1002/ps.724.
19. Das, B. K., Mukherjee, S.C. Toxicity of cypermethrin in *Labeo rohita* fingerlings, biochemical and enzymatic and hematological consequences. *Comp. Biochem. Physiol C. Toxicol Pharmacol*. 2003; Jan 134(1): 109 121.
20. Dimitri, R.A., Gabal, M.A., Saleh, N., Effect of aflatoxin ingestion in feed on body weight gain and tissue residues in rabbits. Mycoses, 1998; 41: 87_/91.
21. Douwes, J., Thorne, P., Pearce, N., Heederik, D., Bioaerosol health effects and exposure assessment: progress and prospects. Annals of Occupational Hygiene, 2003; 47: 187-200.
22. Fink-Gremmels J, Vet. Q. 1999; 21: 115.

23. Fink-Gremmels, J., Malekinejad, H., 2007. Biochemical mechanisms and clinical effects associated with exposure to the mycoestrogen zearalenone. In: Morgavi, D.P., Riley, R.T. (Eds.), *Fusarium* and their toxins: Mycology, occurrence, toxicity, control and economic impact. Anim. Feed Sci. Technol.
24. Forgacs, J., Carll, W.T., 1962. Mycotoxicoses. In: Brandly, C.A., Jungherr, E.L. (Eds.), Advances in Veterinary Sciences. Academic Press, New York, pp. 274–382.
25. Fung, F., Clark, R.F., Health effects of mycotoxins: a toxicological overview. J. Toxicol. Clin. Toxicol. 2004; 42: 217–234, PubMed.
26. Guengerich, F.P., Forging the links between metabolism and carcinogenesis. Mutation Research, 2001; 488: 195_/209.
27. Halstensen, A.S., Nordby, K.C., Elen, O., Eduard, W., Ochratoxin A in grain dustestimated exposure and relations to agricultural practices in grain production. Annals of Agricultural and Environmental Medicine, 2004; 11: 245-254.
28. Hsieh, D.P., Beltran, L.M., Fukayama, M.Y., Rice, D.W., Wong, J.J., Production and isolation of aflatoxin M1 for toxicological studies. J. Assoc. Off Anal. Chem. 1986; 69: 510–512.
29. Huwig, A., Freimund, S., K¨appeli, O., Dutler, H., Mycotoxin detoxication of animal feed by different adsorbents. Toxicol. Lett. 2001; 122(2): 179–188.
30. Imaoka, S., Ikemoto, S., Shimada, T., Funae, Y., Mutagenic activation of aflatoxin B1 by pulmonary, renal, and hepatic cytochrome P450s from rats. Mutation Research, 1992; 269: 231_/236.
31. Jarvis, B.B., Chemistry and toxicology of molds isolated from water-damaged buildings. Mycotoxins and food safety. Adv. Exp. Med. Biol., 2002; 504: 43–52.
32. Jiang, Y., Jolly, P.E., Ellis, W.O., Wang, J.S., Phillips, T.D., Williams, J.H., Aflatoxin B1 albumin adduct levels and cellular immune status in Ghanaians. Int. Immunol. 2005; 17: 807–814.
33. Krysinska-Traczyk, E., Perkowski, J., Dutkiewicz, J., Levels of fungi and mycotoxins in the samples of grain and grain dust collected from five various cereal crops in eastern Poland. Annals of Agricultural and Environmental Medicine. 2007; 14: 159-167.
34. Lewis, L., Onsongo, M., Njapau, H., Schurz-Rogers, H., Luber, G., Kieszak, S., Nyamongo, J., Backer, L., Dahiye, A.M., Misore, A., Decoct, K., Rubin, C., Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in Eastern and Central Kenya. Environ. Health Perspect. 2005; 113: 1763–1767.
35. Linaker, C., Smedley, J., Respiratory illness in agricultural workers. Occupational Medicine, 2002; 52: 451-459.
36. Liu, H.Y., Peng, S.Q., Wu, M., Effect of butenolide on lipid peroxidation in vivo and in vitro. Bulletin of Academy Military Medical Sciences (in Chinese). 1999; 23: 182–184.
37. London, W.T., Evans, A.A., McGlynn, K., Buetow, K., An, P, Gao, L., et al., Viral, host and environmental risk factors for hepatocellular carcinoma: a prospective study in Haimen City, China, Intervirology, 1995; 38: 155–161.
38. Mannon J, Jonson E, New Sci. 1985; 105: 12.
39. Martinek, R.G. Review of methods for determining urea nitrogen in biological fluids. J. Amer. Med. Tech., 1969; 31: 678.
40. Morgavi, D., Riley, R.T. 2007. An historical overview of field disease outbreaks known or suspected to be caused by consumption of feeds contaminated with Fusarium toxins. In: Morgavi, D.P., Riley, R.T. (Eds.), *Fusarium* and their toxins: Mycology, occurrence, toxicity, control and economic impact. Anim. Feed Sci. Technol.
41. Ngindu, a., Johnson, b.k., Kenya, p.r., Ngira, j.a., Ocheng, d.m., Nandwa, h., et al., 1982. outbreak of acute hepatitis caused by aflatoxin poisoning in kenya. Lancet, 8285; 1: 1346–1348, pubmed.
42. Osweiler, G.D., Mycotoxins. Contemporary issues of food animal health and productivity. Vet. Clin. North Am., Food. Anim. Pract. 2000; 16: 511–530.
43. Peng, T., Li, L.Q., Peng, M.H., Liu, Z.M., Liu, T.W., Guo, Y., Xiao, K.Y., Qin, Z., Ye, X.P., Mo, X.S., et al., Evaluation of oxidative stress in a group of adolescents exposed to a high level of aflatoxin B1–a multi-center and multi-biomarker study. Carcinogenesis, 2007; 28: 2347–2354.
44. Pestka, J.J., 2007. Deoxynivalenol: toxicity, mechanisms and health risks. In: D.P. Morgavi, R.T. Riley (Eds), *Fusarium* and their toxins: Mycology, occurrence, toxicity, control and economic impact. Anim. Feed Sci. Technol.
45. Pfohl-Leszkowicz, A., Manderville, R.A., Ochratoxin A: an overview on toxicity and carcinogenicity in animals and humans. Mol. Nutr. Food Res. 2007; 51: 61–99.
46. Pineiro, M., Mycotoxins: natural toxins of agricultural and health significance. Forum Nutr., 2003; 56: 409–412.
47. Quist, C.F., Bounous, D.I., Kilburn, J.V., Nettles, V.F., Wyatt, R.D., The effect of dietary aflatoxin on wild turkey poults. Journal of Wildlife Diseases, 2000; 36: 436_444.
48. Raisuddin, S., Singh, K.P., Zaidi, S.I., Paul, B.N., Ray, P.K., Immunosuppressive effects of aflatoxin in growing rats. Mycopathologia, 1993; 124: 189_194.
49. Reboux, G., Piarroux, R., Mauny, F., Madroszyk, A., Millon, L., Bardonnet, K., Dalphin, J.C., Role of molds in farmer's lung disease in Eastern France. American Journal of Respiratory and Critical Care Medicine, 2001; 163: 1534-1539.
50. Richard J.L., Payne G.A, Mycotoxins: Risks in Plant, Animal and Human Systems, Council for Agricultural Science and Technology, Ames, IA, USA, 2003; R139.

51. Rotter, B.A., Thompson, B.K., Lessard, M., Trenholm, H.L., Tryphonas, H., 1994. Influence of low-level exposure to *Fusarium* mycotoxins on selected immunological and hematological parameters in young swine. Fundam. Appl. Toxicol. 23: 117–124.
52. Sava V, Reunova O, Velasquez A, Harbison R, Sanchez-Ramos J. Acute neurotoxic effects of the fungal metabolite ochratoxin-A. Neurotoxicology, 2006; 27: 82–92.
53. Sell, S., Xu, K.L., Huff, W.E., Kabena, L.F., Harvery, R.B., Dunsford, H.A., Aflatoxin exposure produces serum alphafetoprotein elevations and marked oval cell proliferation in young male Pekin ducklings. Pathology, 1998; 30: 34_/39.
54. Shephard, G.S., Mycotoxins worldwide: current issues in Africa. In: Barug, D., Van Egmond, H., Lopez-Garcia, R., Van Ossenbruggen, T., Visconti, A. (Eds.), Meeting The Mycotoxin Menace. Wageningen Academic, Wageningen, 2004; 81–88.
55. Smith, J.E., Aflatoxins. In: D'Mello, J.P.F. (Ed.), Handbook of Plant and Fungal Toxicants. CRC Press, Boca Raton, FL, 1997; 269-285.
56. Smith, J.E., Solomons, G.L., Lewis, C.W., Anderson, J.G., 1994. Mycotoxins in Human Nutrition and Health. Brussels: European Commission CG XII.
57. Squire, R.A., Ranking animal carcinogens: a proposed regulatory approach. Science, 1981; 214: 877–880.
58. Stark, A.A., Threat assessment of mycotoxins as weapons: molecular mechanisms of acute toxicity. J. Food Prot. 2005; 68: 1285–1293.
59. Theumer, M.G., Lo´pez, A.G., Masih, D.T., Chulze, S.N., Rubinstein, H.R., Immunobiological effects of fumonisin B1 in experimental subchronic mycotoxicoses in rats. Clinical and Diagnostic Laboratory Immunology, 2002; 9: 149-155.
60. Ubagai, T., Tansho, S., Ito, T., Ono, Y., Influences of aflatoxin B1 on reactive oxygen species generation and chemotaxis of human polymorphonuclear leukocytes. Toxicol. In Vitro, 2008; 22: 1115–1120.
61. Voss, K.A., Haschek, W.M., 2007. Fumonisins: toxicokinetics, mechanism of action and toxicity. In: Morgavi, D.P., Riley, R.T. (Eds.), *Fusarium* and their toxins: Mycology, occurrence, toxicity, control and economic impact. Anim. Feed Sci. Technol.
62. Wiedow, O., Meyer-Hoffert, U., Neutrophil serine proteases: potential key regulators of cell signaling during inflammation. J. Intern. Med. 2005; 257: 319–328.
63. Wilson. D.M., Payne. G.A., Factors affecting aspergillus flavus group infection and aflatoxin contamination of crops, in: D.L. Eaton, J.D. Groopman (Eds.), The Toxicology of Aflatoxins; Human Health, Veterinary and Agricultural Significance, Academic Press, San Diego, CA, 1994; 309– 325.
64. Wu, F. 2007. Measuring the economic impacts of *Fusarium* toxins in animal feeds In: Morgavi, D.P., Riley, R.T. (Eds.), *Fusarium* and their toxins: Mycology, occurrence, toxicity, control and economic impact. Anim. Feed Sci. Technol.
65. Wu, F., 2006. Economic impact of fumonisin and aflatoxin regulations on global corn and peanut markets. In: Barug, D., Bhatnager, D., van Egmond, H.P., van der Kamp, J.W., van Osenbruggen, W.A., Visconti, A. (Eds.), The Mycotoxin Factbook. Food & Feed Topics. Wageningen Academic Publishers, The Netherlands.
66. Wybenga, D. R. V. J. Pileggi, P. H. Dirstine,' and John Di Giorgio. Direct Manual Determination of Serum Total Cholesterol with a Single Stable Reagent. *Clinical Chemistry*, 1970; 16: 12.
67. Yaling, W., Tongjie, C., Guozhong, L., Chunsan, Q., Huiyong, D., Meiling, Y., Bert-Andree, Z., Gerd, S., Simultaneous detection of airborne aflatoxin, ochratoxin and zearlaenone in poultry house by immunoaffinity column and high performance liquid chromatography. Environ. Res. 2008; 107: 139–144.
68. Yeragi, S.G., Rana, A. M. and Koli, V.A. Effect of pesticides on proteinmetabolism of mudskipper *Boelophthalmus dussumieri*. J *Ecotoxicol Environ. Monit.* 2003; 13(3): 211 214.