Effect of Aflatoxins on Haematological Profile of Albino Rats (*Rattus norvegicus*)

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Abstract— Aflatoxins are the most dangerous mycotoxin, and are known to be toxigenic carcinogenic, mutagenic, and teratogenic to man and different species of animals. This research is focused on understanding their effect, immediately after intake, using Albino rats as experimental animal. This will help to effectively mitigate the effect of these toxins on their targets. Twenty Albino rats were used, ten fed with Aflatoxins mixed with their feed, while ten others were fed with feed without Aflatoxins, and all were sustain as such for ten days. Eight rats were randomly selected and sacrificed and their blood analyzed for effect of Aflatoxins poisoning. Significant difference was observed comparing the haematological profile of the poisoned rats with the ones not poisoned.

Index Terms—

1 INTRODUCTION

Aflatoxins are mycotoxins produced by certain species of Aspergillus (*Aspergillus flavus* and *Aspergillus parasiticus*) that have been shown to be toxigenic, carcinogenic, mutagenic, and teratogenic to different species of animals (Gupta, 2011). They are toxins produced by moulds that grow in poorly stored nuts, seeds, and legumes. Mycotoxins are secondary metabolites that have no biochemical significance in fungal growth and development. Mycotoxins of greatest public health hazards include Aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins, tremorgenic toxins, and ergot alkaloids. (Ayoub *et al.*, 2011). It is also common in cereal grains like, Maize, Sorghum, Rice, Wheat, etc. (Theddeus, 2009).

Aflatoxins-producing members of *Aspergillus* are common and widespread in nature. They can colonize and contaminate grains before harvest or during storage (Campos *et al*., 2008). . Host crops are particularly susceptible to infection by *Aspergillus* following prolonged exposure to a high-humidity environment, or damage from stressful conditions such as drought, a condition that lowers the barrier to entry (Campos *et al*., 2008).

The native habitat of *Aspergillus* is in the soil, decaying vegetation, hay, and grains undergoing microbiological deterioration. It also invades all types of organic substrates whenever conditions are favourable for its growth.

1.1 Types of Aflatoxins

Various types of Aflatoxins exist, but the major types are; Aflatoxins B1 and B2, Aflatoxins G1 and G2, and Aflatoxins M1. The M1 are found in the milk of animals that are fed with feed contaminated with AFB1 (Iheanacho, 2015). The toxins are very carcinogenic, especially AFB1, which is most abundantly produced and has been, classified among the Group 1 human carcinogens, along with B2, G1, and G2, while M1 is classified as Group 2B human carcinogens. (Krishnamurthy and Shashikala, 2006). Aflatoxins reputation as a potent poison explain why it has been adopted for use in bioterrorism (Klich, 2004

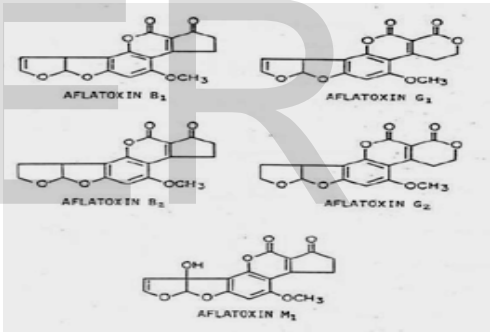

Figure 1: Chemical structures of aflatoxins B1, aflatoxins B2, aflatoxins G1, aflatoxins B2,
aflatoxins G2 and aflatoxins M1. (Source: David K. Okello et al., 2010)

1.2 Exposure and Susceptibility to Aflatoxins

Aflatoxicosis is primarily a hepatic disease. The susceptibility of individual animals to Aflatoxins varies considerably depending on species, age, sex, and nutrition (Bankole and Adebanjo, 2003) In fact; Aflatoxins cause liver damage, decreased milk, and egg production, recurrent infection because of immunity suppression (eg. Salmonellosis), in addition to embryo toxicity in animals consuming low dietary concentrations. While the young of a species are most susceptible, all ages are affected but in different degrees for different species (Bankole and Adebanjo, 2003). Clinical signs of aflatoxicosis in animals include gastrointestinal dysfunction, reduced productivity, reduced feed utilization and efficiency, anemia, and

jaundice. Nursing animals may be affected because of the conversion of aflatoxins B1 to the metabolite aflatoxins M1 secreted in milk of dairy cattle.

Exposure to aflatoxins is widespread in West Africa, probably starting in the uterus, and blood tests have shown that very high percentage of West Africans are exposed to Aflatoxins. In a study carried out in the Gambia, Guinea Conakry, Nigeria and Senegal, over 98 % of subjects tested positive to aflatoxins markers (Bankole and Adebanjo, 2003)

There is evidence suggesting that, aflatoxins may well be a factor in the HIV epidemic and in malaria incidence (Williams *et al*., 2004). Low levels of aflatoxins exposure require continuous consumption for several weeks to months in order for signs of liver dysfunction to appear.

1.3 Haematological Profile Analysis as a diagnostic tool

Blood tests are often used in health care to determine physiological and biochemical states, such as disease, mineral content, pharmaceutical drug effectiveness, and organ function. Haematology, is the branch of medicine concerned with the study of the cause, prognosis, treatment, and prevention of diseases related to blood, it is a branch of internal medicine that deals with the physiology, pathology, etiology, diagnosis, treatment, prognosis, and prevention of blood-related disorders.

Aflatoxicosis causes several defects in organs and tissues, decrease in growth rate, increase in death rate, immunosuppression, anemia, and increase in coagulation time and deteriorates lipid, carbohydrate, and protein metabolism (Celik et al., 2000; Raju and Devegowda, 2000) As a result of toxic effect of aflatoxin, biochemical and haematological parameters have been reported to be changed importantly. In chronic and subclinical aflatoxicosis case, changes in biochemical and haematological parameters occur before clinical symptoms develop (Arawind et al., 2003; Donmez and Keskin 2008). Significant changes in serum biochemical and haematological parameters are seen in aflatoxicosis cases, and these can assist in the diagnosis of toxicities (Basmacioglu et al., 2005; Donmez and Keskin 2009)

2 MATERIALS AND METHODS

2.1 Sources of experimental animal

Rattus norvegicus popularly known as Wister rat or albino rat was sourced from Parasitology Laboratory, Nigerian Defence Academy, Ribadu campus, Kaduna.

2.2 Treatment of experimental animal

Twenty rats were grouped into two categories 5 per cage, making four cages altogether. All the rats were fed ad-libitum (feed available for them to eat at any time they want) with poultry feed (broiler finisher) with maize husk in ratio of 50 % w/w and allowed to acclimatize for three weeks. The first category was fed with the groundnut having 1428.88ppb aflatoxins B1, 249.48ppb aflatoxins B2, 820.53ppb aflatoxins G1, and 162.5ppb aflatoxins G2 mixed with their feed (in ratio of 10:90) for ten days while the other category was only fed with normal feed (broiler finisher with maize husk in ratio of 50 % w/w).

Group A: Aflatoxins and groundnut mixture.
Group B: These were not fed with aflatoxins and groundnut mixture and. They were only fed with the normal feed (Broiler finisher mixed with maize husk)

The animals fed with mixture of Aflatoxins infested groundnut and feed (ratio 10:90) were sustained on the ration for 10 days and observed for another 10 days for effect of Aflatoxins

Eight rats per treatment were randomly selected (four from each cage) and taken to Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, for haematological analysis.

2.3 Animal sacrifice for Haematology Analysis

At the end of twenty days of intoxication, the rats were exposed to chloroform vapour in a desiccator and the animals sacrificed by cervical decapitation (Leelavinothan and Ramalingam, 2013; Aissi *et al*., 2016).

2.3.1 Collection of blood

Blood was collected by cervical decapitation into heparinized sample tubes, and part of it was taken for haematological analysis and the other part was used for digestion (Leelavinothan and Ramalingam, 2013.)

2.3.2 Haematological Measurement

Haematological test on nine parameters were analyzed namely; Pack cell volume (PCV), Heamoglobin (Hgb), White blood cell (WBC), Red blood cell (RBC), Total protein (Total Pro), Neutrocytes (Neut), Lymphocytes (Lymph), Monocytes (Mono), Eosinophils (EOS). There are two categories of treatments; One was poisoned with aflatoxins, while the other was not., four rats were randomly selected, and were given clinic numbers (A1, A2, A3, A4 to B1, B2, B3 and B4 respectively). One group was not up to four rats as an albino rat from this treatment died in transit to Ahmadu Bello University Zaria.

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The PCV was measured using heamatocrit reader, the Hgb by heamoglobinometer and the White blood cell (WBC), Red blood cell (RBC), measured by the use of blood cell analyser machine (Felman *et al*., 2000; Baker *et al*., 2001).

2.4 Statistical analysis

Analysis of variance (ANOVA) at 1 % significant value ($p \leq 0.01$) was used to evaluate some of the results. Some of the data were presented in Tables and in form of Histogram.

3 RESULTS

3.1 Visual observation of the rats

The pictures in plates 1-3 below help to illustrate the deleterious effect of Aflatoxins on the rats. A young albino rat from group B (treated with P. guajava) was found to become gaunt and developed fluffy hair, this situation deteriorated until it died. Other rats manifested this trait but this particular one was the worst. Another diseased condition was observed from a Rat in the negative control after poisoning with aflatoxins which eventually died.

3.2: Heamatology profile

The table of haematological test as seen below is showing the nine parameters that were analyzed namely; Pack cell volume (PCV), Heamoglobin (Hgb), White blood cell (WBC), Red blood cell (RBC), Total protein (Total Pro), Neutrocytes (Neut), Lymphocytes (Lymp), Monocytes (Mono), Eosinophils (EOS). The treatments from the *A. digitata* and Negative control have three rats each as an albino rat from each of those treatments died before they were sacrificed for heamatological and histopathological analysis.

TABLE 1: Haematological parameters and their values for the albino rats after aflatoxins exposure and treatment with medicinal plants

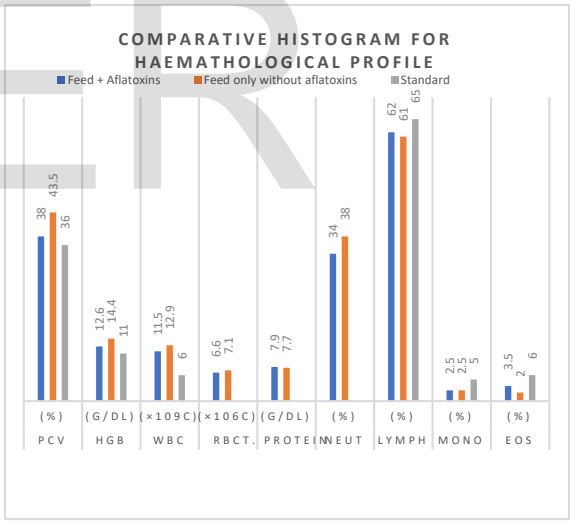
TREATMENT	RATS CLINICNOS	PCV (%)	HGB (g/dl)	WBC (×10⁹c)	RBC (×10⁶c)	T. PROTEIN (g/dl)	NEUT (%)	LYMPH (%)	MONO (%)	EOS (%)
Feed + Aflatoxins	B1	27	9.0	8.4	5.0	7.2	40	60	--	--
	B2	42	14.0	12.9	7.0	7.5	30	64	02	04
	B3	45	15.0	13.1	7.7	9.0	32	62	03	03
Feed only without aflatoxins	A1	46	15.3	13.8	6.9	8.1	35	60	03	02
	A2	40	13.0	12.0	6.8	7.0	30	64	02	03
	A3	43	14.3	12.2	7.2	7.6	37	60	--	03
	A4	45	15.0	13.4	7.4	8.3	40	60	--	--

KEY: Pack cell volume (PCV), Heamoglobin (Hgb), White blood cell (WBC), Red blood cell(RBC), Total protein(Total Pro), Neutrocytes (Neut), Lymphocytes (Lymp), Monocytes (Mono), Eosinophils (EOS)

DISCUSSION

HEAMATOLOGICAL ANALYSIS

Analysis of blood parameters like haematological profile has been a vital tool in determining health status in man and animals. In most cases before clinical signs of ill health became conspicuous, haematological profile would have shown marked deviation from optimal standard. The serum activities of alanine transaminase (ALT), and alkaline phosphatase (ALP) have been recognized as sensitive serological indicators in the impairment of the hepatic tissues and biliary system, and the serum level of total protein (TP) is the indicator of protein synthesis (Abdel-Wahhab, M.A. and Aly, S.E. 2005). Therefore, Yu et al (2015) in their study stated that, the increased serum aspartate aminotransferase (AST) activity observed in the chickens fed diets containing aflatoxins indicates that at least certain damage occurred in the liver. This is because AST, originally located in the cytoplasm, is released into the blood system only when hepatic structural integrity is affected. The following hematological parameters were analyzed namely; Pack cell volume (PCV), Heamoglobin (Hgb), White blood cell (WBC), Red blood cell (RBC), Total protein (Total Pro), Neutrocytes (Neut), Lymphocytes (Lymp), Monocytes (Mono), Eosinophils (EOS). The bar chart above shows marked variations in the haematological profiles of poisoned rats and the rats that were not poisoned.



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