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Effect of coleus forskohlii extract on reduced red blood cell parameters induced by aflatoxin B_1 in albino rats

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Contamination of foodstuff and animal feeds by aflatoxin producing molds is a recurrent global public health problem. Aflatoxin B1 is the most potent and carcinogenic of all aflatoxin subtypes. It has been implicated in the pathogenesis of conditions such as cell carcinoma, malnutrition, immunosuppression, bone marrow depression and growth retardation. This study aimed to demonstrate the effect of coleus forskohlii roots extract on reduction of red blood cell parameters induced by aflatoxin B1 in albino rats. Eighteen male albino rats were divided randomly into three equal groups. The first group was given tap water free from mycotoxins. The second group was toxicated with intraperitoneal injection of aflatoxin B1. The third group was aflatoxicated in the same manner plus coleus forskohlii extract administration. A venous blood sample was obtained for measurement of red blood cell parameters. Result: Aflatoxicated rats in the second group demonstrated a significant decrease in total erythrocyte count, hemoglobin concentration, and packed cell volume. Rats treated with coleus forskohlii extract showed normal values of red blood cell parameters. This study revealed that the depression of red blood cell paramters induced by aflatoxin B1 in albino rats was antagonized by coleus forskohlii roots extract.

Key words: Aflatoxin B_1; Red blood cell parameters, coleus forskohlii roots extract.

INTRODUCTION

Aflatoxins are group of approximately 20 related fungal metabolites produced primarily by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*, which are responsible for the decomposition of plant materials (Cortes etal.,2010). Aflatoxins are classified on the basis of immune-fluorescent properties. The four major naturally known aflatoxins include AFB_1, AFB_2, AFG_1, and AFG_2 where the “B” and “G” refer to the blue and green fluorecnet colors produced under ultra violet light on thin layer chromatography plates, while the subscript numbers 1 and 2 indicate major and minor compounds, respectively (Bennet and Klich, 2003). The World Health Organization classifies AFB_1 as class 1 carcinogen (WHO.2000). The various food products contaminated with aflatoxins include cereals like maize, sorghum, pearl millet, rice, and wheat; oil seeds such as groundnuts, soybean, sunflower, and cotton; spices like chillies black pepper, coriander, turmeric, and zinger; tree nuts such as almonds, pistachio, walnuts, and cocoa nut; and milk and milk products (Lopez et al., 2002). Aflatoxin contamination is influenced by high humidity, high temperature, insect and rodent activity, and inadequate drying of the crops. Globally, about 4.5 billion people are at risk of chronic exposure to aflatoxins (Williams et al., 2004). Very high concentrations of aflatoxins are most often found in nutritive seeds such as maize, nuts, and cereals

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grains in Africa and rice in China and South Asia (Sudakin, 2003). This contamination can occur at any stage of food production from pre-harvest to food processing (Bankole and Adfebanjo, 2003). Measurements of aflatoxin levels in food crops have been conducted in some West African countries such as Ghana where eight samples of maize from 15 (53%) processing sites revealed contamination with fumonisins and aflatoxins (Turner etal.,2005). One study, which collected samples from major processing sites in Accra, Ghana, reported aflatoxin levels that ranged from 2-662 ug/Kg (Kopodo et al., 2006). The latter quantities far exceed the United States Department of Agriculture's (USDA) regulatory limit of 22 ppb (Awuah and Kpodo ,1996). Aflatoxins are highly lipophilic and readily absorbed from gastrointestinal tract (Agag,2004). Aflatoxins are mainly metabolized by the liver to a reactive epoxide intermediate or hydroxylated to become the less harmful aflatoxin M (Wu and Khlangwiset, 2010). In humans and susceptible animal species, aflatoxins especially AFB1 are metabolized by cytochrome P450 (CYP450) microsomal enzymes to aflatoxin-8,9- epoxide, a reactive form that bind to DNA and to albumin in the blood forming adducts and hence causing DNA damage (Wild and Montesano, 2009). AFB1 binds to albumin to form AFB1-lysine adducts (AL-ALB), which persists for up to 2-3 months or longer in the blood (Jolly et al., 2006). The aflatoxins were initially isolated and identified as the causative agent in Turkey X disease that caused necrosis of the liver in 1960, and over 100,000 Turkeys died in USA and England and the death was attributed to the consumption of mold-contaminated peanut meal (Otsuki et al., 2002). Aflatoxins have been implicated in the pathogenesis of conditions such as primary liver cell carcinoma, malnutrition, bone marrow depression, immunosuppression, and growth retardation (*Fung and Clark .,2004*). Aflatoxins have been linked to anemia in pregnancy (Shuaib et al., 2010) and alteration in erythrocytes during chronic aflatoxicosis in rabbits (Verma and Raval, 1992). Aflatoxin causes hematopoietic suppression and anemia, decrease in total erythrocyte count, packed-cell volume and hemoglobin concentration (Reddy and Waliyar 2012) as well as toxicity to red blood cells (Verma and Raval 1991; Ditert et al., 1992). Aflatoxin is known to produce hemolytic anemia by decreasing the circulating mature erythrocyte and consequently the spleen appears congested because of an unusual high concentration of inorganic iron and debris from the circulation (Verma and Raval 1992).

MATERIAL AND METHODS

Eighteen male albino rats of three to four months old weighing100-150 grams were used in this study. The animals were housed in cages under controlled conditions of temperature 22 ± 2 °C., humidity of 60-70%, twelve hour light dark cycle, and provided with a balanced diet. The animals were acclimated for two weeks prior to the experiment. The rats were divided randomly into three equal groups each consists of six rats. The first group was given tap water free of mycotoxins. This group represents a negative control group. The second group is the group of aflatoxicated rats which were given single doses of aflatoxin B_1 intraperitoneally at a rate of 1.5 ppm (Lu and Li 2002). This group represents a positive control group. Aflatoxin B_1 was obtained from Sigma Chemical Company (Sigma Aldrich Corporation, P.O. Box 14508, St. Louis, Missouri, 63178, USA). The third group given aflatoxin B_1 as in group two plus coleus forskohlii extract in a daily dose of 50 mg/Kg (Uchida et al., 2005). Coleus forskohlii extract was obtained according to Kanne et al (2015).

A venous blood sample was taken from postcaval vein using a 28-gauge heparinized insulin syringe for measurement of red blood cell parameters using a hematological analyzer (culture Micro Diff II Coulter Electronic Ltd, USA). The obtained data were computerized and analyzed for significance using analysis of variance ANOVA (F-test) according to Snedecor and Cochran (1982).

RESULTS AND DISCUSSION

In Table 1 Mean± standard deviation in the same column followed by different letters are significant different at $P<0.05$. using LSD(least significant difference). five animals per group. PCV= Packed cell volume .Hb=haemoglobin . RBCs=red bloodcells. CFE= Coleus forskohlii roots extract. AFB_1= aflatoxins B_1.

Aflatoxicosis is a condition caused by aflatoxins in both humans and animals. There are two general forms of aflatoxicosis, acute primary aflatoxicosis produced when moderate to high levels of aflatoxins are consumed. Specific acute episodes of disease may induce hemorrhage, acute liver damage, edema, alteration in digestion, absorption, and/or metabolism of nutrients, and possibly death(Peraica,et al., 1999). The chronic primary aflatoxicosis results from ingestion of low to moderate levels of aflatoxins (Bbosa etal.,2013), inducing teratogenic effects associated with malformations, mutagenic effects that lead to change in genetic code, altering DNA, and genocarcinogens (Wangikar etal.,2005). The present study revealed that the reduction of red blood cell parameters (packed cell volume, total erythrocyte count, and hemoglobin concentration) induced by AFB_1 in Albino rats is antagonized by coleus forskohlii extract., that is demonstrated, by elevation of red blood cell parameters in group of aflatoxicated rats treated with coleus forskohlii extract .This result is consistent with the results of Forgacs and Carll (1962) and Verma and Raval, (1992), they recorded that aflatoxin was often capable of reproducing the hemorrhagic syndrome in poultry and alteration in erythrocytes

Table 1: Effect of Coleus forskohlii roots extract (CFE) on red blood cell parameters of aflatoxicated rats

Parameters Group	PCV (%)	Hb (g/dL)	RBCs ($x10^6$ /µL)
Control	33.2±8.2b	13.9±0.25a	5.18±0.06b
AFB_1 group	20.5±2.1d	9.5±0.38c	3.01±0.02d
AFB_{1+} CFE group	43.0±4.3a	13.7±0.27a	5.60±0.11a

in rabbits. Furthermore Shuaib et al.,(2010) who found association between anemia and aflatoxin B_1 in humans, suggesting that aflatoxins may cause inhibition of hematopoiesis, prompt defective hematopoiesis, increase destruction of red blood cells, or a combination of all three (Verma and Raval,1991) . It has been established that aflatoxins and its metabolites as well as the generated reactive oxygen species (ROS) have a deleterious effects on the bone marrow and blood cells as well as induction of cancers on the hemopoietic system in bone marrow and lymphoid organs where, blood cells and blood components are produced (Halliwell,2007).In humans it is not clear how aflatoxins may cause anemia, however, the fact that it acts as a toxin suggests that it may, like other toxins, cause anemia by a hemolytic process (Dugdale et al., 2009). The accumulation of evidence suggests that aflatoxins may cause DNA damage, mutations, and suppress bone marrow functions (Verma, 2004). The mechanism of this toxicity is thought to occur by a pathway, which involves the metabolism of aflatoxins into epioxide intermediates that go on to bind DNA and RNA. These intermediates interfere with DNA-dependent RNA polymerase, thereby inhibiting RNA and protein synthesis (Williams et al., 2004). These effects of aflatoxins may explain, at least partially, their possible role in causation of anemia. The human kidneys are highly vulnerable to toxic agents exposure. Where the kidneys receive 20% to 25% of resting cardiac output. In addition the kidneys have very high oxygen consumption, since tremendous amount of water and solutes reabsorbed in the nephron (Harriet, 2003). AFB_1 has been reported to produce different aspects of renal lesion in experimental animals with features of megalocytosis in the proximal renal tubules as well as loss of microvili (Al-Mahalaway, 2015). Erythropoietin is the major growth factor for the proliferation and differentiation of committed erythroid progenitor cells. It is produced primarily in the kidney by the endothelial, peritubular, and epithelial tubular cells (Maxwell etal., 1990) in response to hypoxia, and there is inverse relationship between erythropoietin in serum and urine and the level of tissue oxygenation (Krantz and Jacobson, 1970). Mechanism of action by which aflatoxins aggravated pathogenesis of anemia could involve down regulation of erythropoietin activity (Reddy etal., 1987). Coleus forskohlii roots extract antagonized the effect of aflatoxin on bone marrow by its antioxidant effect (Rao et al., 2006).This may be due to Coleus forskohlii functions by binding to and activating adenylylcyclase, leading to increased production of cyclic adenosine monophosphate, cAMP (Ishikawa,2003),and increased level of this intracellular second messenger has been shown to have anti-inflammatory and tissue protective-effects(Erdogan,etal.,2008).

CONCLUSION

The exact mechanism by which aflatoxins induce anemia remains to be elucidated. This study demonstrates coleus forskohlii roots extract as a potential therapeutic agent for treatment of anemia.

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