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Regular Article

Cytotoxic and Genotoxic Effect of Aflatoxin B1

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The aims of study is to determine cytogenetic and genotoxic effect of aflatoxin B1 in vivo, cytogenetic tests are used in studying include chromosome malformation, cell proliferation and plast index test in bone marrow cell in Wight albino rats, the results show that toxin causes Varity in chromosome malformation and increased the rate of it, so it increased in cell proliferation and plast index value compare with negative control.

Keywords: chromosome malformation, plast index, cell proliferation

Introduction

Mycotoxins are important toxins produced by different strains of fungi. Mycotoxins include variety of toxins, one importance of these are aflatoxin B1 (AFB1) that produce from *Aspergillus app*. In humans and animal lab species it has been reported as a potent toxin that causes hepatotoxic, mutagenic, genotoxic, and hepatocarcinogenic (Massey et al., 1995 ;McLean and Dutton, 1995). Chronic exposure to low levels of aflatoxins is one of the major risk factors in the etiology of human hepatocellular carcinoma in several regions of the world (Preston and Williams ,2005). AFB1 is the most potent of the known AFs , and is a classified within class 1 of human carcinogens according to IARC (IARC, 1993). AFB1 is inhalation with contaminated food activated by cytochrome P450 enzyme system to produce a highly reactive intermediate, AFB1-8,9-epoxide, which subsequently binds to nucleophilic sites in DNA forming 8,9-dihydro-8-(N7guanyl)-9-hydroxy-AFB1adduct, figure (1) , which is regarded as a critical step in the initiation of AFB1-induced carcinogenesis (Sharma and Farmer, 2004; Preston and Williams, 2005). In addition, the AFB1-associated mutagenesis was suggested to represent a plausible cause for the higher chromosome malformation in Chinese Hepatocellular Carcinomas, when compared with European primary liver carcinomas (Pineaua et al ,.2008) In contrast, AFB1, human carcinogen and the most potent genotoxic agent, is mutagenic and produces chromosomal aberrations, micronuclei, sister-chromatid exchange, unscheduled DNA synthesis, and chromosomal strand breaks as well as forms adducts in rodent and human cells (Wang and Groopman, 1999).

Materials and Methods

Aflatoxin B1 isolation: AFB1 was isolated from contaminated rise by cultured it on Potato dextrose agar for 5 days in 28 C°, then *Asperagillus terreus* colonies was purify by re-culture it for 4 time, then one colony from pure culture was transfer to potato dextrose broth for 14 days in 28 C° to isolate AFB1, broth was filtered more than one, the filtration solution that contain toxin AFB1 kept in 4 C° until detection by TLC.

Detection AFB1: toxin was detection by thin layer chromatography (TLC) using methanol: chloroform (97: 3) (v/v) as mobile phase with stander toxin isolation (Trucksess, *et al* 1984).

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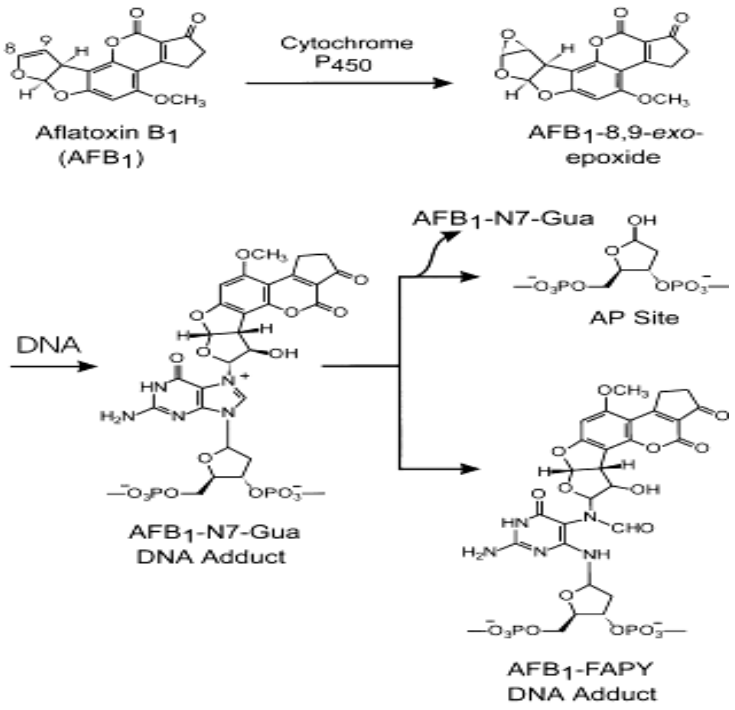
Experimental design : use Wight albino rat (300±50) in age 13±2 weeks , animal divided in to 3 groups

1- Animal treated by distal water as negative control.
2- Animal treated by cyclophosphamide drug 10 mg\kg for 15 days as positive control
3- Animal treated by AFB1 1ml\kg for 15 days.



Cytogenetic assay

Karyotyping preparation: this assay was performed according to Tolliver and Robbins (1991) with modifications ; animal was treated with chloroform for 5 min. then it fixed it, femur bone was removed and cleaned it to remove all muscle , then it ends was cutting to wash bone marrow in test tube, washing steps repeat more time by hypotonic solution, tubes was incubated in 37 c for 20 min with shaker, after this it was centrifugation 2000 rpm for 2 min, supernatant was removed and sediment was shacked it to re suspension cells, than fixative solution was added in 4 c (2 ml to tube) centrifugation again 2000 rpm for 2 min supernatant remove gently , this step repeat 2 time , in the last fixative step half of supernatant removed and add 1 ml of fixative solution , left tubes on flat table , for 1 min. then dropped cell suspension on slid in high 100 cm , left slide dry then stained it by gemsa stain.

Cytogenetic assays include:

Chromosome malformation test; this assay performed according to Tolliver and Robbins (1991).

Cell proliferation test; this test performed according to Ghosh *et al* (1991).

Plast index test performed according to Ghosh *et al* (1991).

Statistical analysis; statics performed by SPSS using (ANOVA-one way) at $p<0.05$.

Results

The results show that AFB1 caused changes in all cytogenetic assays that performed in studying as show below:

1- Detection AFB1, toxin isolation from contaminated rise, was AFB1 when it detected by TLC with stander AFB1 and AFB1 as show in figure (5).
2- Chromosome malformation test; table (1) and figure (2) revealed effect of AFB1 on bone marrow chromosomes of rats, it causes different types of aberration that clarify in figure (6), all types of aberration were significant increased in AFB1 treated rats compare with negative control, the total of aberrations was 53.65% while in negative control was 3.49.
3- Cell proliferation test; AFB1 causes significant increase in cell
4- Proliferation as show in figure (3) and table (2).
5- Plast index; AFB1 also causes significant increase in plast cell as in figure (4) table (2).

Table (1) Types of chromosome malformation in rats bone marrow cells treated by aflatoxin B1 for 15 days.

Me±Se * significant at p<0.05

Treatment	Chromatid break	Aneuploidy	Ring	Analytical	Sticky	Breaking
Negative control	1.70±0.10	0.80±0.23	0.66±0.35	0	0	0.33±1.33
Aflatoxin B1	7.33±2.72	14.33±3.17*	2.00±1.15*	11.33±4.25*	14.00±2.64	4.66±1.76*
Cyclophsphomid	16.80±1.89*	19.4±2.91*	6.93±0.96*	16.66±3.47*	6.93±0.96*	14.80±2.07*

Table (2) Cell proliferation and plast index value of rat's bone marrow cells treated by aflatoxin B1.

Me±Se * significant at p<0.05

Treatment	Cell proliferation	Plast index
Negative control	5.16±0.42	6.56±1.54
Aflatoxin B1	7.56±0.88*	7.96±1.24
Positive control	1.76±0.27	13.66±1.50*

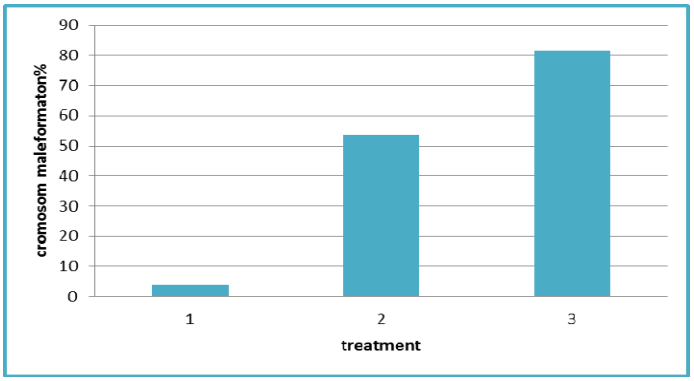


Figure (2) Percentage of total chromosome malformation in bone marrow cell. 1-NC, 2-treated by aflatoxin b1, 3- positive control.

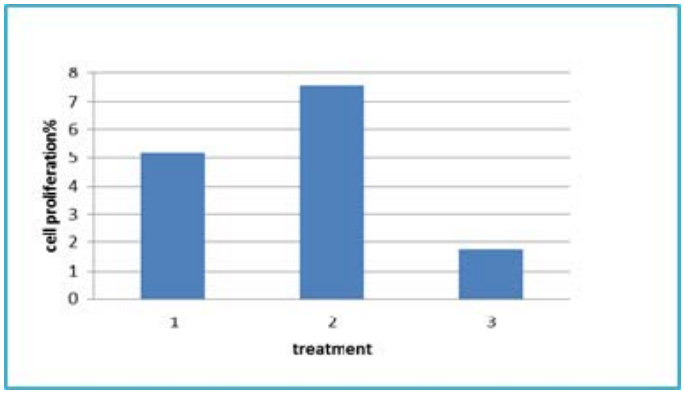


Figure (3) Cell proliferation value of bone marrow cells. 1, negative control; 2, aflatoxin B1; 3, positive control (CP).

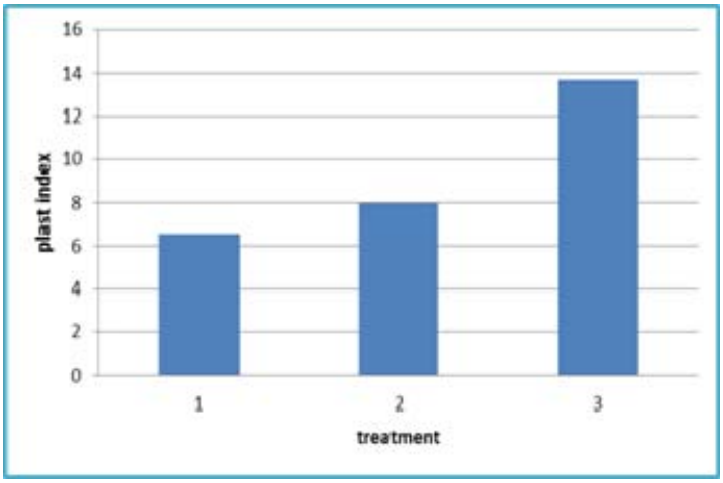


Figure (4) Plast index value of rat's bone marrow cell. 1- NC, 2- treated by aflatoxin b1, 3- positive control.

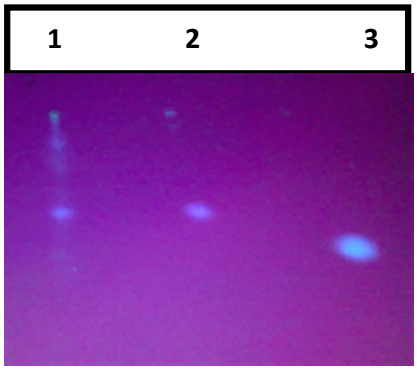


Figure (5) Detection aflatoxin B1 by TLC using chloroform: methanol (3:97 v/v) as mobile phase. 1, aflatoxin B1 (sampel); 2, stander AFB1; 3, stander AFB2.

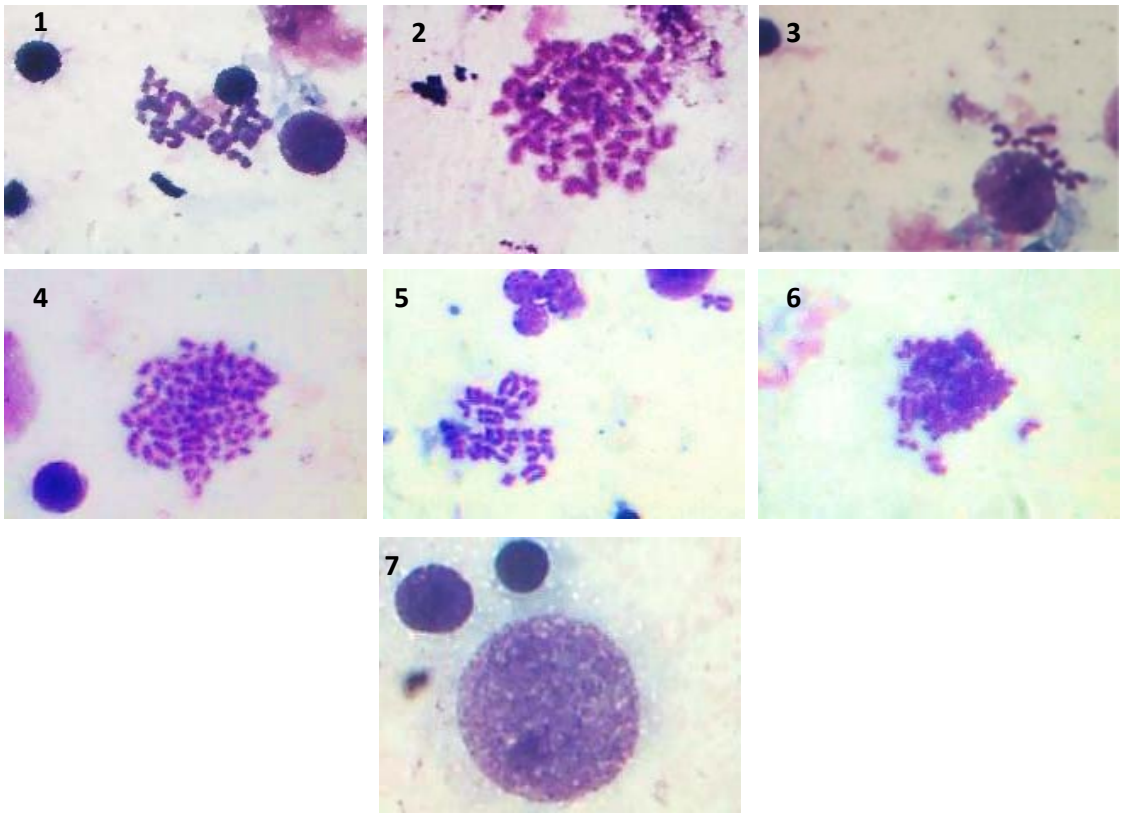


Figure (6) Types of chromosome malformation in rats bone marrow cells that treated by AFB1. 1,sticky; 2, ring; 3,aneuploidy; 3,breaking; 4,chromatid break; 5,analytical; 6,plast cell.

DISSCUTION

The results show that AFB1 causes variety types chromosome malformation in bone marrow cells, these types were chromatid break, aneuploidy, analytical, sticky, and breaking and ring chromosomes, because AFB1 is potent genotoxic agent (Foster and Eisenstadt, 1983) and causes DNA damage in plant and animals lab, so it causes mutation in germ cell (Bilgrami and Sinha 1988; Yakicier *et al* 2001). Other studying suggesting that sub lethal dose of AFL causes chronic toxicity and low chronic dose causes cancer in some organs and inhibition in DNA, RNA and protein (Busby and Wogan 1985; Mclean and Dutton 1995). Darwish *et al* (2011) improved that Aflatoxin is genotoxic in bone marrow and spermatocyte cell and had cytotoxic effect in both cell type, when they study chromosome structural features they found that Aflatoxin causes structural and numerical changes in somatic and germ cell. Some researcher found that some types of aflatoxins such as B2, G1 and G2 effected on mice chromosomes. Theumera (2010) suggesting that effect of Aflatoxin result from increasing generation intracellular reactive oxygen species ROS which causes different damage in cell component especially in DNA, RNA, and protein. Also AFB1 causes micronuclei in rat's bone marrow cell, and different lysis in DNA of some organs such as liver, kidney and spleen (Al-Terehi, 2012).

In plants Agar and Alpsay (2005) illustrated that AFG1 causes increased in chromosome aberration, cell proliferation and total protein in crop plant. Molecular study revealed that AFB1 causes mutation in DNA, Levy *et al* (1992) illustrated that four of seven mutational hotspot in human xeoderma pigmentosum cell observed in *supF* gene of the

Ps189 shuttle vector are at AGG sequence; three of these hotspot show different reactivities toward AFB1 . IN bacterial system AFB1 induced GC→TA transvertion mutation and induced SOS response, also induced mutation in *lacI* gene in bacteria, and *ras* gene mutation in rainbow trout liver, in human induced mutation in *Ha-ras* proto oncogene (Lawrence *et al* 1990; Loeb *et al* 1986).

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