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Research Article

The role of vitamin A on the genotoxic effects induced by aflatoxin B1 by chromosomal aberrations assay in rats

Iman Abd El-Moneim Darwish[1] and Sahar Abd El Razik Mosallam[2]

[1,2]Zoology Department, Women' s College for Arts, Science and Education,
Ain Shams University, Cairo, Egypt
*Corresponding author: *imandarwish73@yahoo.com*

Abstract

The current study was conducted to evaluated the ability of vitamin A for the prevention of genotoxicity induced by aflatoxin B_1 (AFB_1) using the chromosomal aberrations assay in rats. Five groups of rats were treated for 14 weeks and included the control group, AFB (1)-treated group, dimethylsulfoxide (DMSO) treated group, dimethylsulfoxide and vitamin A and vitamin A in combination with aflatoxin B_1. Treatment with DMSO or aflatoxin B_1 had a significant effect on structural chromosome aberrations in rats. However, DMSO + vitamin A slightly decrease the structural chromosomal aberrations in bone marrow cells. AFB_1 combined with vitamin A decreased the level of mitotic metaphase aberrations, although they still higher compared with the control group. The frequency of total structural chromosomal aberrations increased from 5.33 % (excluding centromeric attenuation) in the control to 30.33 % in bone marrow of rats treated with aflatoxin. All types of chromosomal aberrations induced by aflatoxin were substantially reduced by vitamin A. As can be seen, vitamin A pretreatment of cells caused protection against the toxicity of aflatoxin.

Keywords: Aflatoxin B_1 (AFB_1), Dimethyl sulfoxide (DMSO), vitamin A (Vit A), structural chromosomal aberrations (SCA).

Introduction

AFB_1 is the prominent one among the four aflatoxins B_1, B_2, G_1 and G_2 of corn in China (Gao et al., 2011). Aflatoxins are mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* that can contaminate human and animal foods, including corn, wheat, rice, peanuts, and many other crops resulting in the illness or death of human and animal consumers (Mardani et al., 2011).

The decrease of aflatoxin contamination might be made practical through reducing wheat storage duration and controlling humidity (Taheri et al., 2012). The biological control of bacteria against aflatoxin was showed a wide distribution in Chinese peanut main producing areas (Wang et al., 2012). Eom et al. (2013) suggested that dietary AFB_1 exposure might be associated with a risk of gastric cancer.

Approximately 4.5 billion of the world`s population is exposed to aflatoxin-contaminated food, particularly in low-income countries (Hamid et al., 2013).

Qi et al. (2013) noticed a number of genetic and gene expression alterations were found to be associated with HBV and AFB_1-related (hepatitis C virus) HCC. Adedara et al. (2014) indicated that exposure to AFB_1 had significant reproductive health implications for consumers of contaminated products even under conditions of low dietary toxin levels.

The high incidence of breakage rate was paralled by an increased sister chromatid exchange (SCEs) rate in human chromosomes by aflatoxin B_1 (el-Zawahri et al., 1977). A close correlation in response to sister chromatid exchange induction by AFB_1 was showed in

Chinese hamster ovary (CHO) and human cells (Thomson and_Evans, 1979). The induction of sister chromatid exchanges (SCEs) in the hepatic tumor cell line (HTC) by AFB_1 was inhibited by estradiol, a known inhibitor of microsomal activating enzymes (Dean et al., 1980). The chromosomal damage in Chinese hamster bone-marrow cells was showed due to treatment with aflatoxin B_1 (AFB_1), aflatoxin G_1 (AFG_1) and platulin (PA) (Korte, 1980).

Aflatoxin B_1 exhibited Hypodiploid chromosome sets, and destruction of the chromosomes of the primary hepatomas in Wistar rats (Gasiorowski and Zawirska, 1981). The mutagenic activity of aflatoxin B_1 on human chromosomes was higher than that of G_1 and although chromosomes 2, 11, 19, and 20 were the most affected by aflatoxin B_1, chromosomes 1, 2, 3, 4, and 5 were the most affected by aflatoxin G_1 (el-Zawahri et al., 1990).

Vitamin A minimised the frequency of aflatoxin B_1-induced clastogeny in both mitotic and meiotic chromosomes in mice (Sinha and Dharmshila, 1994). A possible interaction between P53 gene mutation and 4q loss were indicated in the pathogenesis of hepatocellular carcinoma in the people's Republic of China, regions with a high and an intermediate exposure to aflatoxin, respectively (Rashid et al., 1999). Individual risk factors were associated with distinct genetic aberrations, although changes in 1q gain appear common to all tumor samples obtained from regions that differed in aflatoxin exposure (Wong et al., 2000). Aflatoxin B_1-associated mutagenesis represented a plausible cause for the higher chromosome instability observed in Chinese hepatocellular carcinoma, when compared with European primary liver carcinomas (Pineau et al., 2008).

A technique for efficiently generating large chromosomal deletions in the Koji molds *Aspergillus oryzae* and *Aspergillus sojae* was established by using a Ku 70-deficient strain and a bidirectional marker (Takahashi et al., 2008). Moreover, vitamins A, C and E could effectively inhibit AFB_1-induced sister chromatid exchange (SCE) in cultured human lymphocytes (Alpsoy et al., 2009). AFB_1 induced significant alterations in chromosome aberrations test, DNA fragmentation assay, malondialdehyde (MDA) level and the heat shock proteins Hsp 70 and Hsp 27 expressions (Brahmi et al., 2011). The sister chromatid exchange and micronucleus rates were decreased due to co application of borax and aflatoxin as compared to the group treated with AFB_1 alone (Turkez et al., 2012). Recently, AFB_1 increased the frequencies of sister chromatid exchange and the level of malondialdehyde (MDA) and decreased the activities of superoxide dismutase (SOD), glutathione (GSH) and glutathione peroxidase (GPx) (Ceker et al., 2013). Kalinina et al. (2013) noticed that the loss of chromosome 18q was associated with early onset of tumours and with the patient's place of birth in Russian populations exposed to aflatoxin B_1. The present study aimed to evaluate the effect of vitamin A against aflatoxin B_1 induced structural chromosomal aberrations in bone marrow cells of rats.

Materials and Methods

Animals: Female rats (40-50gm) were fed with pellet and water ad libitum and maintained in controlled atmosphere of 12-h dark / light period and 22 ± 2^0C temperature.

Chemical and Treatment: Aflatoxin B_1 (AFB_1) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, USA).

AFB_1 (3 ug/ml each in benzene: acetonitrile (98 : 2) was dissolved in 10% DMSO (diluted in distilled water). Female albino rats were orally administered aflatoxin B_1 in a dose mimicking human exposure condition i.e. at 0.05 micrograms/kg body weight/day for 14 weeks. Vitamin A (retinol) was orally administered along with the toxin in double (132 1u/kg body weight/day) the human equivalent therapeutic dose. This dose was further calculated for the rat, according to Paget and Barnes (1964), to be 0.062 1u/kg b.w. Thirty female rats were divided into five equal groups as follows: a control group (received distilled water), a group treated with DMSO, a group treated with dimethyl sulfoxide and vitamin A, a group treated with AFB_1 alone or in combination with vitamin A, respectively.

Chromosomal assay: Rats were sacrificied by cervical dislocation and the bone marrow was obtained from both femora. Routine preparations of metaphases were made and stained with Giemsa. Chromosomal aberration was calculated by counting 50 metaphase spreads / animal and expressed in percentage (OECD, 1997).

Statistical Analysis: One-way analysis of variance was performed and variant groups were determined by means of the Dunnett t-test, P value was assumed to be significant at 0.05.

Results

The structural chromosomal aberrations

For each rat, 50 metaphases were selected at random for microscopic examination. Most of them had 42 chromosomes per metaphase. In control animals, chromosome aberrations did occur, but their frequency never exceeded 5.33±3.93% and centromeric attenuation were the most chromosome anomalies detected in this control (Table I). In the untreated bone marrow cells approximately 1.33% of the chromosomal aberrations were of the exchange type.
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Table (I): Description and frequencies of various types of structural abnormalities detected in mitotic metaphase chromosomes from bone marrow of rats treated with aflatoxin B1 and vitamin A

Experimental group	Number of examined metaphases	Structural chromosomal aberrations/300 cells								Total SCA including c a Mean ± S.D%	Total SCA Excluding c a Mean ± S.D%
		c a	R t	e to e ass	del	dic	ac f	r	g		
Control	**300**	19	1	4	6	1	2	-	2	35 11.66±3.44	16 5.33±3.93
DMSO	**300**	62	8	10	20	14	9	5	-	128 42.66±3.50***	66 22±6.32***
DMSO + Vitamin A	**300**	63	8	4	10	7	3	4	1	100 33.33±8.64***	37 12.33±2.65*
Aflatoxin B_1	**300**	94	14	25	20	14	10	3	5	185 61.66±9.33***	91 30.33±5.57***
Aflatoxin B_1 + Vitamin A	**300**	42	2	4	15	8	3	9	1	84 28±5.21***	42 14±3.57**

c a= centromeric attenuation R t = Robertsonian translocations e to e ass= end to end association del= deletion dic= dicentric ac f =acentric fragment r= ring g = gap
*=p≤0.05 **=p≤0.01 ***=p≤0.001 SCA=Structural Chromosomal aberrations Dunnett t-test treat one group as a control, and compare all other groups against it.

DMSO caused a 4.12 fold increase in chromosomal aberrations over that observed in the negative controls. A statistically significant increase in the frequency of chromosome aberrations was noticed by treatment of rats with DMSO used as a vehicle.
The mean percentage of metaphases with chromosome aberrations reached 30.33±5.57(excluding centromeric attenuation) after oral administration of 0.05 ug AFB_1 kg-1b.w. The percentages of total chromosomal aberrations were 22%; 12.33% and 14% after treatment with DMSO, DMSO-vitamin and aflatoxin-vitamin groups, respectively, the difference between the control and each treated group being significant.

Vitamin A, when administered along with the toxin (AFB_1 group + V) decreased the abnormality incidence (14± 3.57%), and brought it slightly less than the DMSO plus vitamin-induced level (12.33 ± 2.65%). Various types of aberrations were observed with the majority being centromeric attenuations. Chromosomes with deleted portions could also be viewed. Such a chromosome had unequal sister chromatids, one of them was markedly shorter than the other. These were noticed in about 6.66% of the examined cells in aflatoxin treated group (Fig. 1).

Chromosome type aberrations, particularly rings, are also observed but at a much lower frequency. Robertsonian translocation was a condition where the two homologous or non-homologous chromosomes were fused at the centromeric region. This type of aberration was speculated in 4.66% of the scored metaphases due to treatment with the fungicide, Fig. (1).

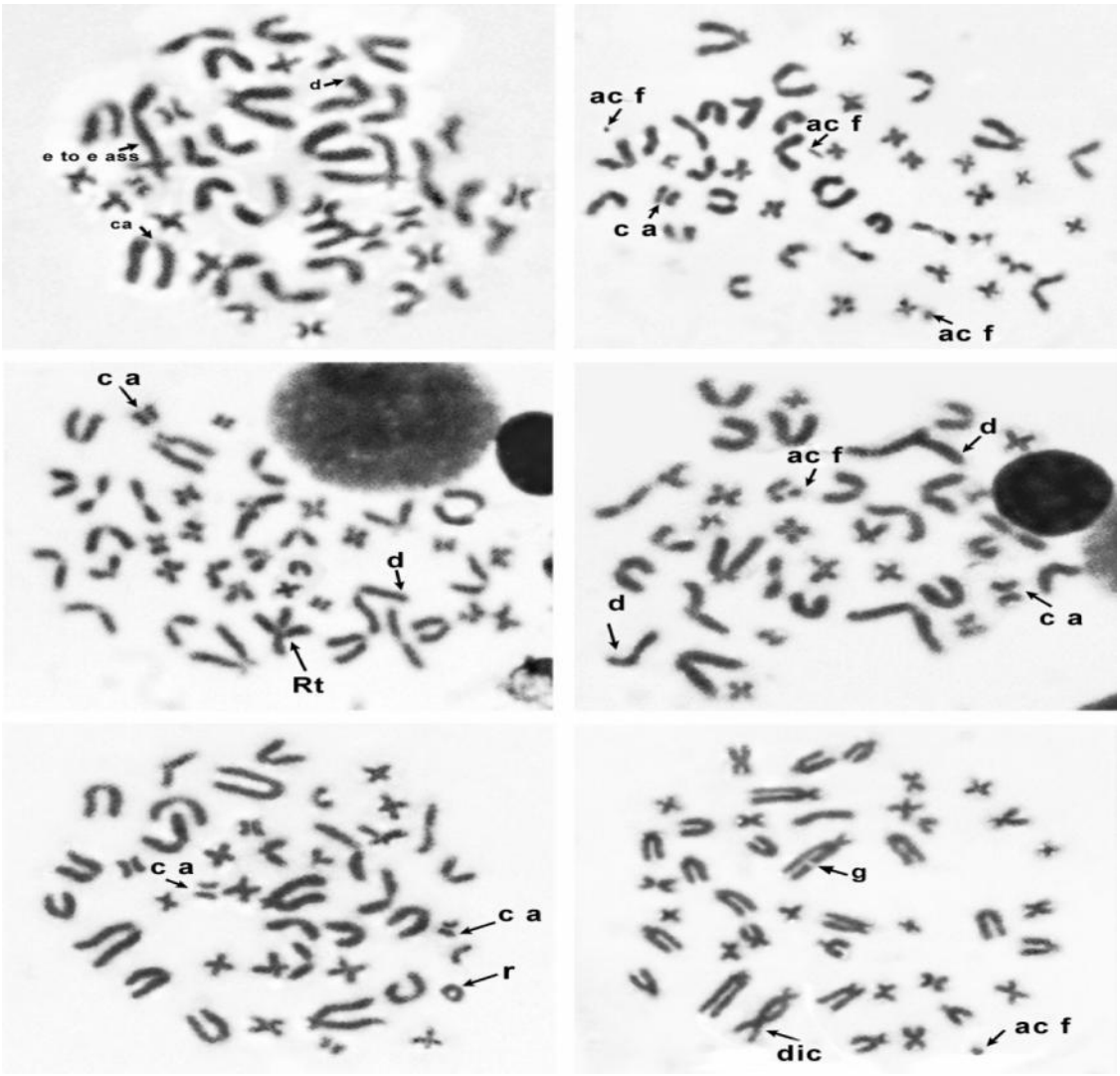

Fig. (1): Structural chromosomal aberrations in female rats treated with oral administration of AFB1 for 14 weeks.

R T=Robertsonian translocation e to e ass =end to end association
d=deletion, dic=dicentric, r=ring, g=gap, ac f=acentric fragment

Chromosomal exchanges were also observed at certain instances. In other occasions, the same metaphase might show more than one type of damage. Gaps and dicentrics occurred in lower frequencies. They were 5 and 14 of the total aberrant chromosomes, i.e. 1.66% and 4.66% of the 300 examined cells in AFB_1 treated group.

An acentric fragment showed up in the form of a small fragment having no centromere. This was noticed in about 3.33% post treatment with aflatoxin. Chromatid break was rare.

Discussion

Aflatoxins are mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* that can contaminate human and animal foods, including corn, wheat, rice, peanuts, and many other crops resulting in the illness or death of human and animal consumers (Mardani et al., 2011).The present work was therefore undertaken with the aim of assessing the antimutagenic effects if any, of vitamin A against the genotoxicity of the chronic exposure to aflatoxin.

Aflatoxin was injected oral gavage into rats with or without the co-administration of 100mg/kg vitamin A. Structural chromosomal aberrations were induced in about 61.66±9.33 & 30.33± 5.57 % (including and excluding centromeric attenuation, respectively) of metaphases examined after the treatment with 0.05ug of aflatoxin B_1 for 14 weeks. Moreover, aflatoxin induced very highly significant increase ($p \leq 0.001$) in the frequencies of CA at the dose studied. This result indicates structure activity relationships in mutagenicity that are in agreement with those of the toxicity and carcinogenicity of aflatoxin B_1.

Aflatoxin probably acted directly or indirectly on the DNA. Although the exact mechanism of DNA damage is not known, several hypothesis have been put forward to explain this, such as inhibition of DNA metabolism, incorporation of nucleotide analogues into DNA, alkylation of DNA, cross-linking of DNA, alterations of the chromosomal structural proteins, lysosomal break down with the release of DNase and alterations in the level of enzymes and co-enzymes (Bradley et al., 1987).

Significant chromosomal aberrations observed at 14 weeks indicate the prolonged action of aflatoxin metabolites on bone marrow cells. The mutagenicity of aflatoxin is mainly dependent on the amount of the drug and its metabolites reaching the target organs and the yield is also dependent on the efficiency of the DNA repair of the affected cells in the test organisms. So the cytogenetic effects showed here might be caused by the action of aflatoxin in mitotic apparatus such as spindle fibres and kinetochores rather than on DNA synthesis which lead to structural anomalies of chromosomes.

However, as compared to aflatoxin treated animals, the abnormal metaphases were always less in aflatoxin plus vitamin A treated group at corresponding time points. Significant decreases in the incidence of abnormal cells were noticed when the aflatoxin and vitamin A were administered concurrently. These findings in turn suggest a definite protective role of vitamin A on chromosomal aberrations induced by aflatoxin.

Retinol (vitamin A) deserves to be included into category of antimutagens, as it also traps free radicals and quenches active oxygen species, the latter two playing important roles in the elaboration of mutagenic radicals and formation of DNA-adducts (Ong and Chytill, 1983).

On the basis of these results it is suggested that human food needs to be supplemented with a suitable dose of vitamin A to combat the menace of aflatoxicosis, the latter being a worldwide hazard (Jelinek *et* al., 1989) due to poor storage and harvesting conditions as well as global trade in food and feeds.

The present results are in conformity with the previous experiments performed using vitamin A and ochratoxin (Dharmshila and Sinha, 1994). Similarly, the present result are in accordance with the results obtained by some investigators after administration of AFB_1 (Sinha and Dharmshila, 1994). They stated that vitamin A minimised the frequency of chromosomal aberrations induced by aflatoxin B_1 in mice.

The predominant chromosomal aberration occurring in bone marrow cells of aflatoxin poisoned rats was of the deletion, centromeric attenuation, end to end association, Robertsonian translocation, gap and usually involves only single chromatids. The predominante aberrations in the primary hepatoma of Wistar rats by aflatoxin B_1 were breaks and deletion of the chromatin material (Gasiorowski and Zawirska, 1981).

Chromosome aberrations of the chromosomal type, such as rings, was not observed in the control group and it was occasionally present in the treated group. Induction of chromatid breaks and fragments elicits the clastogenic potential of aflatoxin which in long term exposure can cause somatic mutations.

The production of a high incidence of gaps, breaks, fragments and other types of chromatid anomalies by aflatoxin indicated that this fungicide must act directly or indirectly on the cell DNA.

Additionally, metaphases contain more than one type of structural chromosomal aberration were highly detected after aflatoxin treatment.

There are many reports available on the effects of this fungicide individually on chromosomes. However, these reports are contradictory in nature and no clear picture was obtained. Accordingly, approximately 27% of the metaphases were caused by chromatid fragment exchange, about 19% by chromatid breaks,

and about 4% were caused by chromosome fragmentation, dicentric chromosomes and questionable aberrations after administration of AFB_1 (Tsutsui et al., 1977).

Proficient removal of adducts was exhibited from both alpha and bulk DNA of monkey cells treated with aflatoxin B_1 in early G_1 or late S/G_2 while those cells treated in early S phase did not remove adducts from either alpha or bulk DNA (Leadon and Hanawalt, 1986).

The molecular mechanism of the aflatoxin B_1-induced mutation is now well understood, and can be summarised by saying that the aflatoxin B_1 forms adducts with DNA bases involving 7-N guanine (Benasutti et al., 1988). Such adducts also produce apurinic and /or apyrimidine sites (AP-sites) along the length of the DNA molecule (Stark et al., 1988), and block DNA replication (Refolo et al., 1987).

Several genotoxicity-suggesting aberrations like sister-chromatid exchanges, recombinations, chromosome aberrations and clastogenic responses also appear due to treatment with AFB_1 (Yu et al., 1990).

AFB_1 is first metabolised (phase 1 metabolism) mainly by the Cytochrome P450 enzyme (CYP450) system found in the microsomes, producing a variety of metabolites such as AFB_1 epoxide and hydroxylated metabolites (AFM_1, AFP_1, AFQ_1 and aflatoxicol). AFB_1 epoxide is a very reactive and unstable metabolite of AFB_1 that will bind to cellular macromolecules like DNA, RNA, lipids and proteins, leading to lipid peroxidation and cellular injury (Stresser et al., 1994).

Similarly, murine models provide further evidence that AFB_1 induces chromosome instability in living organisms (Kaplansky et al., 1997). Interestingly, in mouse models, this instability does not apply to chromosome segments in an indiscriminate way, but instead targets selected chromosomes, e.g., mouse chromosome 12 in lung adenocarcinomas, or human chromosome 13q in HCC (Herzog et al., 2004).

The mechanism of action of AFB_1 begins with metabolic activation of cytochrome p450 to exo-8,9-exposide, of which then produce adducts that modified DNA. Moreover, adducts interaction with guanine will create mutational effects to p53 tumor suppressor gene, causing GC transversion into TA at specific codon 249 (Goldman and Shields, 2003).

The decrease biosynthesis and secretion of protein might be due to formation of aflatoxin adducts with DNA, RNA and protein of mice (Mathuria and Verma, 2008).

AFB_1 stimulates the release of free radical, including reactive oxygen species, which cause chromosomal aberrations (Alpsoy et al., 2009).

Lower concentrations of AFB_1 enhanced cellular proliferation, which was more pronounced in human than in porcine cells, while higher concentrations caused a dose-dependent decrease (Taranu et al., 2010).

A total reduction of AFB_1 induced oxidative damage markers, an anti-genotoxic effect resulting in an efficient prevention of chromosomal aberrations and DNA fragmentation compared to the group treated with AFB_1 alone and decreased p53 as well as its associated genes such as bax and bcI2 in mice treated by AFB_1 and *Cactus cladode* extract (Brahmi et al., 2011).

As mentioned before, the frequencies of sister chromatid exchange (SCE) and malondialdehyde MDA level were decreased and superoxide dismutase, glutathione, glutathione peroxidase level were increased when *Usnea articulata* (UAE) and *Usnea filipendula* (UFE) were added to AFB_1 (Ceker et al., 2013).

Recently, cytochrome P450 2A13 (CYP2A13) was suggested to played an important role in low-concentration AFB_1-induced DNA damage, possibly linking environmental airborne AFB_1 to genetic injury in human respiratory system (Yang et al., 2013).

Conclusion and recommendations: The results show that pure aflatoxin, when administered for 14 weeks, is potent enough to damage mitotic chromosomes. The increased frequency of chromosome aberrations suggests a clastogenic potential of aflatoxin. These observations indicate the *in vivo* susceptibility of mammals to the genetic toxicity potential of aflatoxin.

The present findings also show that the concurrent administration of vitamin A in double human

equivalent therapeutic doses minimised the frequencies of these abnormalities, and brought them slightly above to the incidence observed in the control group of animals. The antigenotoxic and anticarcinogenic potencies of vitamin A may be explain with the mode action of this vitamin, which could be either by interacting with mutagen or by eliminating the damage arise from the carcinogenic agent.

Conflict of Interest statement

There is no conflict of interest statement.

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