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RESEARCH ARTICLE

The toxic effect of aflatoxin on chromosomal aberration and sperm morphology of albino male mice *Mus musculus* L.

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ABSTRACT

Aflatoxins are mycotoxins found as a foodstuff contaminant, but some of them are seen to increase cancer risk. Aflatoxins are the most potent natural toxin recognized. The sperm shape and chromosomal aberration test were used to investigate the effect of aflatoxin on albino male mice. The aim of this research is to assess the cytogenetic and sperm toxic effects of aflatoxin in albino male mice *in vivo*. The doses used were (control, 10, 20, and 30) mg/kg/bw for 24 hours. Our results reveal that aflatoxin can cause a range of chromosomal malformations like chromatid break without fragment, ring chromosomes, dicentric chromosomes, centromeric break, chromatid gap, and fragment chromosomes the abnormalities increased from 5 in control to 62.2 in 30mg/kg. and sperm abnormalities in male albino mice subjected to various doses were hookless, headless, tailless, defective head, long and broad hook, folded head, folded tail, and sperm with double tail, increasing the dose concentration enhanced the rate of it when compared to untreated mice from17.6 in control to 136.4 in the highest dose.

Keywords: Aflatoxins; Chromosomal aberrations; Sperm toxic; Albino mice

INTRODUCTION

Aflatoxins (AF) are produced by many species of *Aspergillus* section flavi, in particular *Aspergillus flavus* and *Aspergillus parasiticus* and *A.nomius,* which are naturally occurring mycotoxins. Aflatoxins represent a major risk to public health and aflatoxins are known to have a strong hepatotoxic and carcinogenic effect (El-Nabarawy et al., 2020), and they're found in moist grains (wheat, rice, barley, corn, protein sources such as rapeseed meal and so on) and nut goods (Fink-Grennels, 1999). Peanuts and cottonseed also contain them (Pitt, 2000). Aflatoxin includes aflatoxin B1, B2, G1, G2, M1, M2 (Sumit et.al. 2010). Aflatoxin B, in particular, has been demonstrated in studies to be a possible carcinogen in several animal species, including rainbow trout and rats (Smela et al., 2001).

Aflatoxin is metabolized in the liver by the group of enzymes known as cytochrome p450 after consumption, where it is transformed to a variety of products of metabolism such as aflatoxicol, Aflatoxin M1, P1, and Q1 depending on the species' genetic susceptibility. There is also a metabolite known as Aflatoxin 8, 9 epoxide, which is produced by a similar way. Because this metabolite can cause mutations by intercalating and forming adducts with the guanine moiety in DNA, the amount of this metabolite determines the species susceptibility (Bondy and Pestka, 2000). Furthermore, the susceptibility of a species to Aflatoxin is largely determined by detoxification systems of its liver, age, genetic make-up, and other factors of nutrition. (Fink-Grennels, 1999).

Also, no animal species has been proven to be resistant to the aflatoxins' acute toxic effects, which are categorized by the International Agency for Research on Cancer as a Category 1 carcinogen. There was a wide variance in the Lethal Dose 50 (LD50) value in animal species tested with single doses of aflatoxins (Maia et al., 2002). Acute aflatoxicosis has been observed in humans from all over the world. (Gong et al., 2002).

Aflatoxin's ability to pass the placental barrier means it can induce genetic abnormalities in prenatal stages (Maxwell et al., 1998). Aflatoxins are fungal secondary metabolites

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made by numerous *Aspergillus* species belonging to the flavi section, that infect a wide range of food products. (Paterson and Lima, 2010). Aflatoxins are a collection of closely related chemicals that have a significant negative influence on the economy and human health (Kensler et al., 2011). The most potent natural carcinogen discovered to date is Aflatoxin B1 (AFB1), (IARC Group), including hepatotoxic, teratogenic, and immunotoxic effects (IARC, 2012).

The liver is AFB1's most important target organ (Meissonnier et al., 2007), CYP1A2 and CYP3A4 process the toxin, resulting in DNA adducts that cause mutations, most notably in the p53 tumor suppressor gene [Eaton and Gallagher, 1994, Oda et al., 2001, and Kensler et al., 2011]. Additionally, AFB1could be metabolized by prostaglandin H synthase chemical. Furthermore, AFB1 could be metabolized mutagenically by prostaglandin H synthase (Battista and Marnett, 1985). The aflatoxin b1 and m1 showed to be toxic to mice kidneys, (Li et al., 2018). AFB_1 can disrupt the blood testis barrier and promoting apoptosis in mice testes, (Huang et al., 2021).

According to the literature we have, most of the study on aflatoxins has been done on somatic cells and there hasn't been much work done in on our region on the impact of aflatoxins on germ cells. So, the goal of this study was to see how increasing doses of aflatoxin affect the chromosomal and sperm morphology of albino mice in Duhok province/Iraq.

MATERIAL AND METHODS

Aflatoxin b1 was utilized at four doses (zero,10, 20, and 30 mg/kg of body weight) in this study. Duhok University's mycology research lab provided this mycotoxin.

The mice were given a single oral treatment with a dose syringe made locally from a disposable syringe (2 ml) and needle.

Animals

BALB/c adult male Swiss albino mice (*Mus musculus*) (8-10) weeks old.

The animals in this study weighed about 30 and 35 grams. All parts of the animal experiment, and parturitions took place in the Animal House of the Biology Department, Faculty of Science of Duhok University, which was kept at room temperature (22 degrees). The mice were fed a regular food as well as water.

Cells of the bone marrow

Each mouse was given 1ml of newly prepared colchicine (0.04%) with intraperitoneal injection at the end of the treatment to stop cell division at metaphase. Animals were killed by cervical dislocation two hours after injection to obtain chromosomes from bone marrow cells, (Evans et al. 1964). The bone marrow slides air dried at room temperature, stained for 10-15 minutes with 2 percent Giemsa stain, and remove the excess stain by washing with phosphate buffer solution. To study chromosomal abnormalities, at least 100 metaphase cells per animal were scored (Sharma and Sharma,1980).

Preparation of sperm

The sperm came from the epididymis and the vas deferens. The epididymis and vas deferens were taken from the reproductive system after the animals were killed and placed in a tiny Petri dish containing normal saline. The epididymis and vas deferens were cut into multiple pieces using a sharp scissor, and the sperms were discharged into a saline solution. The suspension of sperms was spread, dried, and fixed using (3: 1) methanol acetic acid fixative. It was then stained with haematoxylin for 15 minutes, washed with tap water, and dried at room temperature. Each animal had at least 1000 sperm counted to detect abnormalities of sperm morphology. (Wyrobek, 1979).

Statistical analysis

SPSS software was used to conduct statistical analysis. The experiment was conducted in a factorial design with a completely randomized design (CRD). The most important factors were:

The concentrations of dosages were (0.0, 10.0, 20.0, and 30.0 mg/kg/body weight).

Each treatment was carried out five times.

Period= 24 hours

Duncan's multiple range of doses and times was used to evaluate the data after one way analysis of variance (Duncan, 1955).

The significant differences were considered at ($P<0.05$), when the results values were reported as mean with standard deviations.

RESULTS

Effects of aflatoxin on chromosomes in cells of bone marrow of the albino male mice

Table 1 and Fig. 1 show the effects of various aflatoxin treatments on chromosomal aberrations in albino male mice bone marrow cells. A total of 500 metaphase cells were used to score the data (five replicates, 100 metaphase cells from each animal). The aflatoxin generated a variety of cytotoxic consequences after 24 hours of treatment, which are represented by various types of chromosomal abnormalities visible under a light microscope (100x).

Table 1: The Effect of Aflatoxin on Chromosomes Aberrations in Albino Male Mice

Doses	chromatid break without fragment	Ring chromosomes	Dicentric chromosomes	centromeric break	chromatid gap	fragment chromosomes	Total aberrant metaphase
D0=Controls	1 ± 0.316	1 ± 0.316	0.8 ± 0.2	1.6 ± 0.4	0.2 ± 0.2	0.4 ± 0.244	5
D1=10 mg/kg	3 ± 0.316	4.2 ± 0.583	3 ± 0.316	4.4 ± 0.509	1.2 ± 0.374	2.2 ± 0.374	23.4
D2=20 mg/kg	7.2 ± 1.02	5.8 ± 0.86	4.6 ± 1.03	7.8 ± 0.916	4.8 ± 0.374	6.6 ± 0.4	36.8
D3= 30mg/kg	10.8 ± 0.374	12.8 ± 1.158	10.4 ± 0.678	13.8 ± 0.734	6.2 ± 0.374	8.2 ± 0.583	62.2
Total	22	23.8	18.8	27.6	12.4	17.4	127.4

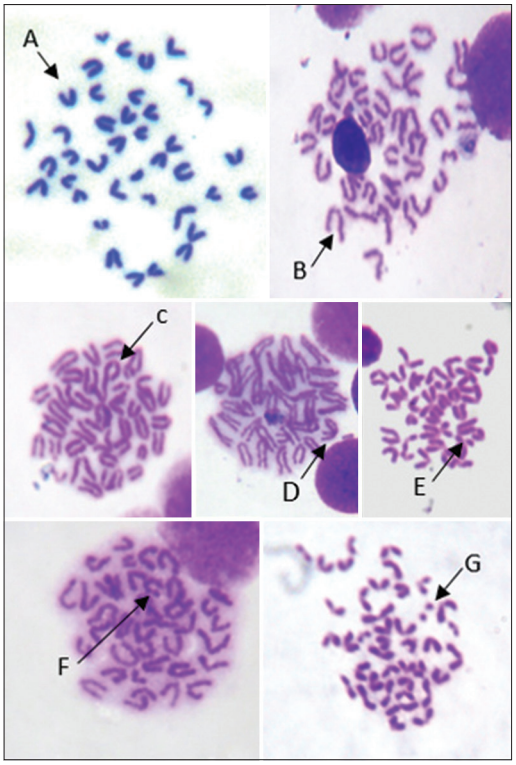

Fig 1. Types of Structural Chromosomal Aberrations Induced by Aflatoxin in Albino Male Mice. A- Normal Chromosomes, B- Chromatid Break without Fragment, C- Ring chromosomes, D- Dicentric chromosomes, E- Centromeric break, F-Chromatid gap, G- Fragment chromosomes.

The data in Table 1 represents the effect of aflatoxin on chromosomes aberrations of the albino male mice. The data were scored from 500 metaphase cells (five replicates, 100 metaphase cells from each animal). Table 4.3 present data that reveal great differences in the total number of abnormalities. The increase of aflatoxin concentration increased the values of abnormalities. Animal exposed to (30mg/kg) showed the highest chromosomal aberrations.

In Table 1 and Fig. 3 it is obvious that most of aberration types were centromeric breaks with values of Control =1.6 ± 0.4, D1=4.4 ±0.509, D2= 7.8± 0.916 and D3=13.8± 0.734. The least values of aberrations were in the chromatid gaps, Control =0.2±0.2, D1=1.2±0.374, D2= 4.8±0.374 and D3=6.2±0.374. there was a high significant effect at ($p<0.001$).

Effects of aflatoxin on Sperm Morphology of the Albino Male Mice

The data were scored from counting of 500 sperms (100 sperm from each animal in each treatment). Different abnormalities are shown in Fig. 3.

Data in Table 2, shows that the highest abnormality in sperm morphology was hookless D0=3.2 ± 0.663, D1=11.8 ± 2.518, D2=19.6 ± 3.234, and D3=27.4 ± 1.6, but the least abnormalities were sperm with double tail D0= 0.2±0.2, D1= 1.2±0.374, D2= 2±0.707 and D3=3.2±0.663. Fig. 4 shows very high significant effect of doses on different types of sperms at ($P<0.0001$) as hookless, headless, tailless, defective head, long and broad hook, folded head, and folded tail, as in Fig. 4. The data in table 2 reveal very high significant differences ($P<0.001$) in the means due the effects of aflatoxin doses. The effects on total abnormal sperms increased constantly with increase of dose concentrations in comparison to non-treated animals. According to Fig. 4 all of the abnormalities in the sperm morphology were significant at $p< 0.001$ except for sperm with double tails.

DISCUSSION

Effects of aflatoxin on chromosomes in cells of bone marrow of the albino male mice

Chromosomal aberrations are the most useful tool for identifying possible genotoxicity of substances because they only count primary lesions of DNA that can not repaired by cell machinery (Evans, 1977 and Blasczyk et al., 2003). Oxidative damage and clastogenic substances are both harmful to bone marrow cells. As a result, models for mutagenicity and/or antimutagenicity screening are routinely used (Umegaki et al., 1997).

According to the current study, the most frequent anomaly as centromeric break. (Knutsen et al., 2010) stated that the chromosomal abnormalities are seen in colorectal cell line. Chromosomal abnormalities in cells of bone marrow of mice, including break of centromere, fragments, Chromatid breaks, chromatid gaps, end-to-end associations, centric fusions, stickiness, deletions, ring chromosomes, dicentric chromosomes, hypoploydoidy, and. chromosomal fusions. gaps were described as achromatic lesions that are in one or both chromatids not surpassing the chromatid width,

Table 2: The Effect of Aflatoxin on Sperm Abnormalities in Albino Male Mice

Doses	Hookless	Headless	Tailless	Defective head	Long and broad hook	Folded head	Folded tail	sperm with double tail	Total abnormal sperm
D0=Controls	3.2 ± 0.663	3.8 ± 0.663	5.8 ± 0.734	0.6 ± 0.244	1.2 ± 0.489	2 ± 0.447	0.8 ± 0.374	0.2 ± 0.2	17.6
D1=10 mg/kg	11.8 ± 2.518	10.8 ± 2.131	9.2 ± 0.583	2.6 ± 0.509	3.6 ± 0.4	4.8 ± 0.374	4 ± 1.225	1.2 ± 0.374	48
D2=20 mg/kg	19.6 ± 3.234	16 ± 1.378	18 ± 1.225	4 ± 0.447	8.6 ± 1.03	11.8 ± 0.734	10.8 ± 0.860	2 ± 0.707	88.8
D3=30 mg/kg	27.4 ± 1.6	23.4 ± 1.631	25.4 ± 1.536	8.4 ± 1.077	14 ± 0.632	21.6 ± 1.364	13. ± 0.836	3.2 ± 0.663	136.4
Total	62	54	58.4	15.6	27.4	40.2	28.6	6.6	290.8

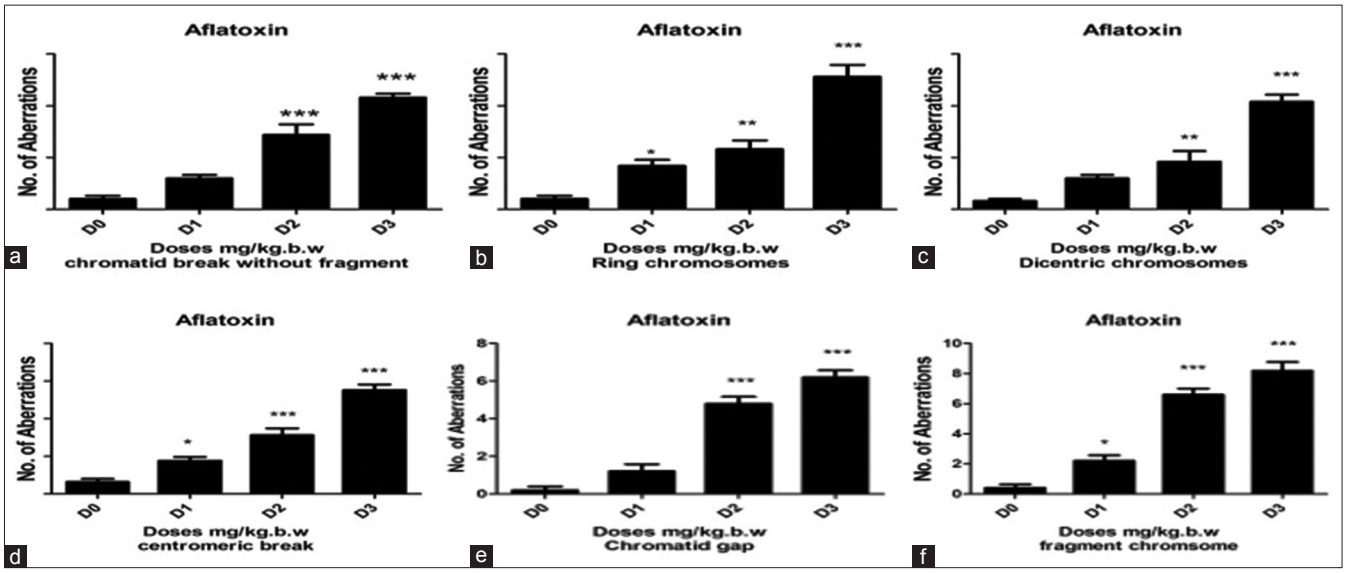

Fig 2. (a-f)The Effects of the Aflatoxin on the Chromosomes Aberrations in Albino Male Mice. *(p< 0.05), **(p< 0.01), ***(p< 0.001).

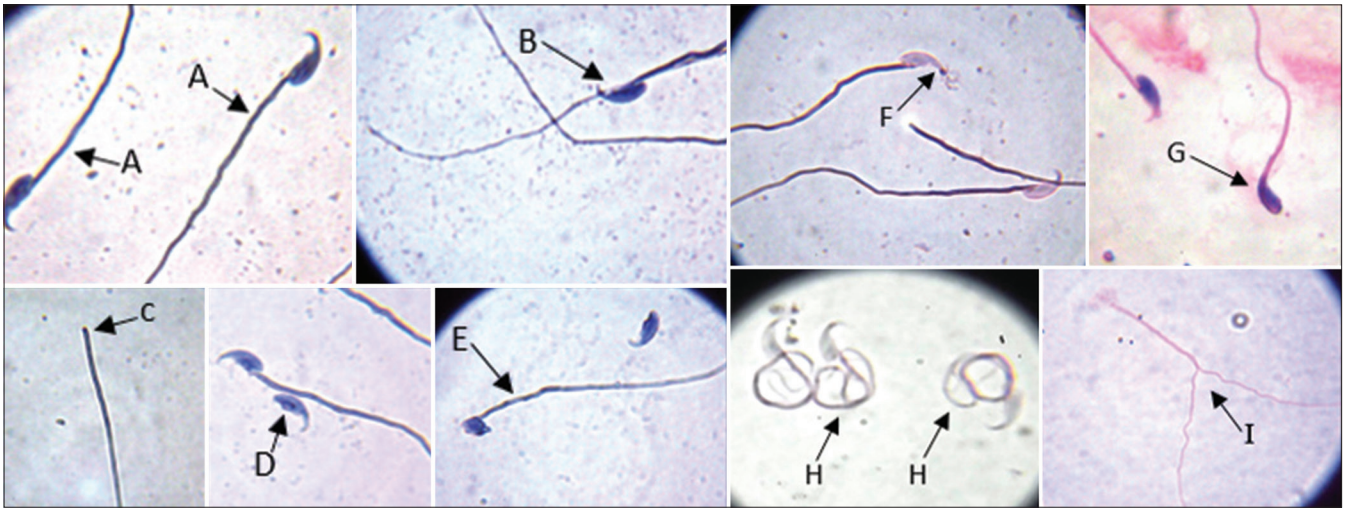

Fig 3. Types of Misshapen Sperms Induced by Aflatoxin in Albino Male Mice. A- Normal Sperm, B- Hookless, C – Headless, D – Tailless, E - Defective Head, F - Long and broad hook, G- Folded head, H- Folded tail, I-sperm with double tail.

while breaks were defined as discontinuities more than the chromatid width, regardless of whether there was dislocation of the distal piece [Ito and Ito, (2001)]. AFB1 was studied for its clastogenic effects on a variety of test animals. They discovered that 0.1 g/g AFB1 induced chromosomal abnormalities in rat bone marrow cells and 1 g/g AFB1 caused chromosomal abnormalities in mice, including deletions, ring chromosomes, and breaks. Aflatoxins caused gaps, breaks, deletions, centromeric attenuations, end mitosis, and polyploidy, as well as end-to-end interactions, fragments, and polyploidy Furthermore, AFB1 generated several sorts of chromosome abnormalities in bone marrow cells of Wight albino rats (dose of 1 mlkg for 15 days), including aneuploidy, chromatid break,

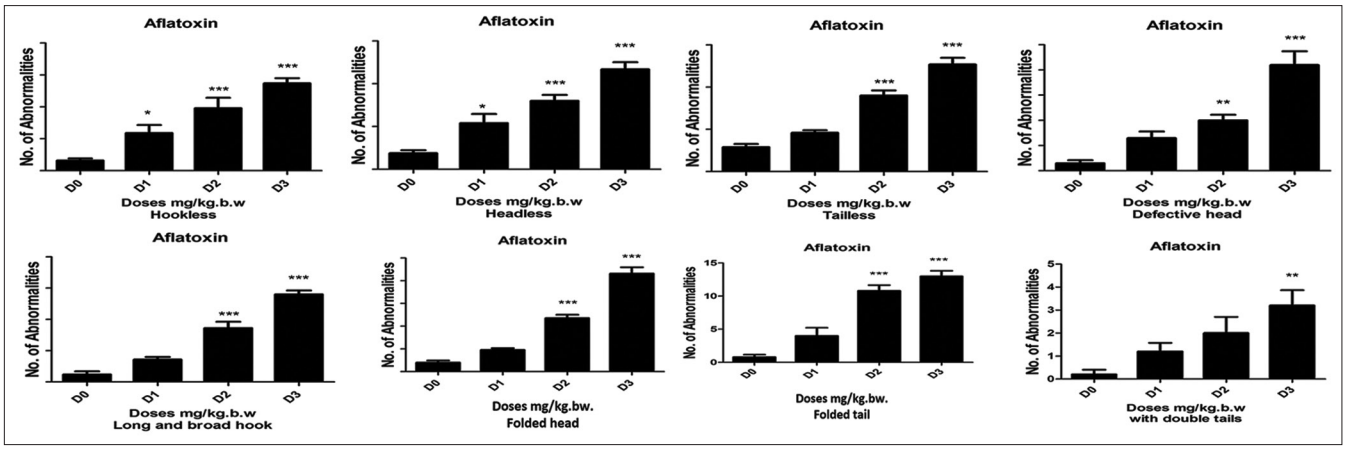

Fig 4. Shows the Effects of the Aflatoxin on the Sperms Abnormality in Albino Male Mice. *(p< 0.05), **(p< 0.01), ***(p< 0.001).

analytical, sticky, ring chromosomes and broken chromatid, according to the reference (Terehi, 2013).

Effects of aflatoxin on sperm morphology of the albino male mice

The observed distortion in morphology of the mice sperm shows that aflatoxin had a negative effect on sperm. Because DNA synthesis happens before the pre-meiotic stages of spermatogenesis and no additional DNA replication occurs during spermatogenesis in the cell cycle, this could have damaged spermatogenesis in its pre-meiotic phase. (Bakare et al., 2005). It could also be related to chromosomal errors that happened during the packing of the genetic material in the sperm head or point mutations that occurred during spermatogenesis.

The findings are consistent with prior publications on carcinogenicity of aflatoxins in a variety of animal species, like rainbow trout and rats (Smela et al., 2001). In animal species treated with only single doses of the aflatoxins, there was also a wide range of LD50 values. (*Maia* et al., 2002).

CONCLUSION

It is concluded from the results of this study that aflatoxin b1 has mutagenic effect on chromosomal structure of albino mice and also causes diverse malformations to its sperm and it is increased with increasing its dose which confirms its genotoxicity, it has adverse effect on both somatic and germ cells which means it effects on the next generation as well.

Author's Contribution

Fana Thikri Ibrahim: methodology, animal handling and experimental procedure, data analysis and results interpretation and writing the original draft. Nasreen Jalal Hussein: results interpretation and discussion, writing the article and designed the figures. Asia Abdulhamid Mohammed Saadullah: provided aflatoxin b**1** from her lab supervised the whole research and reviewed the article. All the authors read and accepted the final manuscript before publishing it.

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