Evaluation of Protective Role of Activated Charcoal Against DNA Damage, Cytogenetic Changes and Reproductive Toxicity Induced by Aflatoxin B1 in Mice

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ABSTRACT

The present study was designed to investigate the protective role of activated charcoal (AC) on aflatoxin B1-induced genotoxicity in male and female mice. Charcoal was supplemented at low (500mg/kg diet) and high (750mg/kg diet) levels to basal diet or basal diet containing 40ug AFB1/kg feed. Sixty adult albino mice (30 males and 30 females) were randomly selected and distributed into 12 groups, 6 groups of males and 6 groups of females (five animals each). Both male and female groups were assigned to six treatments for three weeks. Compared to control group, the results showed that mice (males or females) fed the diet contaminated with AFB1 had significant elevation of the rates of DNA fragmentation, polymorphism proportions of RAPD profiles, the percentages of micro nucleated polychromatic erythrocyte (MNPCE) and frequencies of chromosome aberrations. Moreover, the AFB1 diet caused significant increases of sperm shape abnormalities and significant decreases of sperm count in males as well as it induced significant reduction of each of collected number of oocytes and the rate of matured oocytes in females. Feeding the two sexes of mice on AFB1 diet treated with low or high level of AC resulted in a lower genetic deleterious and enhancement in reproductive parameters. The treatment with high level of AC was more effective for amelioration of the adverse effect of AF than the treatment with low level of such adsorbent. Furthermore, the ameliorative response for genetic parameters due to treatment with AC was clearer in females than males and there were significant differences between the two sexes. In conclusion, the present study proved that AC has antagonistic or protective effect against the genotoxicity of AFB1. The protection degree of AC is probably related to its concentration ratio.

Keywords: Activated charcoal, Aflatoxin B1, Cytogenetic, DNA fragmentation, Mice, RAPD-PCR, Reproductive parameters.

INTRODUCTION

Aflatoxins (AF) are a group of closely related, biologically active mycotoxins, produced by Aspergillus fungi. These toxins frequently contaminate animal and human foods.[1] The main biological effects of aflatoxins are carcinogenicity, immunosupprression, and teratogenicity.[2-4] Moreover, aflatoxins are potent mutagenic food components and have been found to be an inhibitor factor in cell division.[5,3] They are metabolized by the mixed function oxidase system to a number of hydroxylated metabolites and to aflatoxin[8,9] epoxide which binds to DNA, forming covalent adducts[6] and disturbs DNA replication causing chromosomal aberrations.[7, 4]

Management of mycotoxin includes prevention, regulation, monitoring, avoidance, decontamination, detoxification and animal treatments. Safe levels of mycotoxins are difficult to determine.[8,9] Even with excellent management, there may be unavoidably low levels of mycotoxins as a constant concern for potential loss of feedstuffs, increased animal diseases, reduced animal performance and food residues. The use of mycotoxin binders or adsorbents may have the greatest application for routine avoidance of this constant exposure to low level of multiple mycotoxins. Activated charcoal (AC) is an adsorbent widely used in the treatment of wastewater and industrial contaminants by virtue of its high removal capacity and adaptability for a wide range of pollutants.[10] This adsorbent (AC) denotes a material which has an exceptionally high surface area and includes a large amount of microporousity.[10,11] Efficient surface absorption characteristics in addition to the low cost of activated charcoal; it can be used to remove toxic chemicals and pesticides.[12,13] Rotter et al.[14] reported that activated charcoal (50μg) was able to absorb 90% of the ochratoxin A (150μg) contained in 10 ml of citrate-phosphate buffer (PH 7.0).

Concerning the protective role of AC against aflatoxicosis, there are contradicting reports. In one of the first studies to test the concept of mycotoxin binding, activated charcoal was shown to reduce aflatoxicosis in goats.[15] Also, Galvano et al.[16] showed the reduction of aflatoxin residues in milk of cows consuming different sources of charcoal. Teleb et al.[17] found in broilers that the addition of activated charcoal to the AF (30 ppb) contaminated diet at 0.5% level reduced the mortality rate and improved body weight gain and efficiency of feed utilization, also, pathological changes in liver were higher in AF treated group than those found in AF + AC treated group. Moreover, activated charcoal was found to be important in binding with other toxicants such zearalenone and /or deoxynivalenol.[18-20] Also, Azoz and Raafat[11] found in rats that the supplementation of charcoal to lead diet significantly improved the biochemical (AST, ALT, ALP and total protein) and hematological (RBC count, and blood hemoglobin (Hb)

concentration and packed cell volume (PCV)) Parameters as well as significantly decreased the percent of multinucleated polychromatic erythrocytes (MPCE) as compared to rats fed lead diet alone.

In contrast, Kubena et al.[21] reported that the addition of AC to the poultry diet did not show a protective effect against the negative effect of AFB, on some biochemical parameters. Furthermore, in some studies, responses to charcoal with mink[22], turkey poults[23], broiler[24], rats[25] and cows[26], concluded that charcoal had lack of effect on aflatoxicosis. Denli and Okan[27] found that the addition of activated charcoal (2.5mg/kg) to diet containing AFB1 (0.4 or 80 ug AFB1/kg feed) did not ameliorate the negative effects of aflatoxin on broiler such as lower body weight gain, feed inefficiency, significant increase in activity of AST and ALT, significant decrease in concentration of total protein and high significant in liver lesions.

Because of the widespread use of AC in our life and because the contradicting reports about their protection against toxic effect of AFs as well as some animal genetic and reproductive parameters on such subject were not discussed previously. So, further studies are required to evaluate the exact protection role of AC against the toxic effect of AFs in mammalian cells. DNA damage is considered to be main parameters for genetic changes. DNA alterations can be detected by a number of laboratory techniques. Lately, a new DNA approach has been developed to identify and fingerprint living organism, called RAPD Technique (random amplified polymorphic DNA), in which DNA fragments are amplified by polymerase chain reaction (PCR) using a single arbitrary DNA primer.[28,29] Distinct genetic polymorphisms usually observed between different genomes when the amplified products are separated and visualized on agarose gel by electrophoresis. The advantages of the technique are its simplicity and rapidity, to detect extensive polymorphisms, and the fact that only small quantity of DNA is needed.[30]

So, the present study was designed to investigate the protective role of activated charcoal (AC) on aflatoxin B1 – induced genotoxicity in male and female mice. The genotoxicity investigations included DNA damage, cytogenetic changes and reproductive toxicity.

MATERIALS AND METHODS

Chemicals and reagents

Aflatoxin B1 from *Aspergillus flavus* was purchased from Sigma Chemical Co. (St.Louis, MO 631189, U.S.A.). Methanol, trifluoroacetic acid, and sodium chloride, were purchased from Sigma Chemical Co. (St.Louis, MO, U.S.A.). All solvents were of HPLC grade. The water was double distilled with Millipore water purification system (Bedford, M.A., U.S.A.). Potato dextrose agar (PDA) and Yeast extract purchased from Sigma-Aldrich, France.

Fungal strain

Toxigenic strain of *Aspergillus flavus* (ATCC 28542) was obtained from Microbial Research Center, 3 Faculty of Agriculture, Ain Shams University, Cairo, Egypt (MIRCEN).

Adsorb (Synthetic activated charcoal)

Activated charcoal was purchased from company SD Fine-Chem. Limited, product no: 43032.

Specification

Particle size was 300 mesh, methylene blue adsorption (270 mg/g), pH 6-7.5, maximum limits of impurities (moisture 5%, ash 2.5%, acid soluble 2.5% and water soluble 1.5).

Preparation of potato dextrose agar (PDA) and yeast extract sucrose (YES)

The (PDA) was prepared according to the manufacturer's directions by adding 40g of the dehydrated PDA to 1000 ml of distilled water. The culture media was then mixed and autoclaved at 121°C for 15 min. YES culture was carried out according to the method of Munimbazi and Bullerman[31] as following (2% yeast extract and 15% sucrose/liter distilled water) were transferred into 500 Erlenmeyer flask, and autoclaved at 121 °C for 15 min.

Preparation of inoculation

Preparation of spore suspensions: The fungal culture was grown on (PDA) slants at 28 °C for about 14 days or until good sporulation was observed. Spores were harvested by adding growing the *Aspergillus flavus* together with 10ml of sterilized aqueous solution of Tween 80 (0.05%v/v) to culture[32].Spore suspensions were then centrifuged at 20,000 rcf for 5 min. and the supernatants were discarded. The spore concentrations were adjusted to yield a final count of 10^6 spores/ml and the ensuing preparations were used as spore inoculum.

Productions of aflatoxin B1 (AFB1) on yeast extract sucrose

A total of 10 ml of a suspension containing 10^6 spores/ml were added to one liter Erlenmeyer flasks containing with YES medium then incubated at 28 °C for 14 days. After the 14 days the Erlenmeyer flasks were autoclaved at 121°C for 15 min then determination of AFs in liquid medium.

Extraction of AFB1 from liquid media

AFs were extracted according to the method described by El-Banna et al.[33] Extraction was carried out using 20 ml of chloroform (twice with 10 ml media),and homogenization for 3 min in a separation funnel. The chloroform phase was filtered through filter paper Whatman No.3 and concentrated to dryness under a nitrogen stream.

Determination of AFB1 by HPLC

Derivatization: The derivatives of samples and standard were done as follow:100µl of trifluoroacetic acid (TFA)

was added to samples and mixed well for 30 s and the mixture stand for 15 min. 900 µl of water: acetonitrile (9:1v/v) were added and mixed well by vortex for 30 s and the mixture was used for HPLC analysis.

The HPLC system consisted of Waters Binary Pump Model 1525, a Model Waters 1500 Rheodyne manual injector, a Waters 2475 Multi-Wavelength Fluorescence Detector and a data workstation with software Breeze 2. A phenomenex C_{18} (250 X 4.6mm i.d.), 5µm from Waters corporation (U.S.A). An isocratic system with water: acetonitril: Methyanol (240:120:40) was used.[34] The separation was performed at ambient temperature at a flow rate of 1.0 ml/min. The injection volume was 20 µl for both standard solutions and sample extracts. The fluorescence detector was operated at wavelength of 360 nm for excision and 440 nm for emission.

Experimental animals

Males and females of Swiss Albino mice (Mus musculus) weighing 25-30 grams were obtained from the Animal House at the National Research Center, Giza, Egypt. The animals were maintained on standard casein diet and water *ad libitum*. They were housed in a temperature-controlled and artificially illuminated room free from any source of chemical contamination.

Experimental Design

Sixty adult albino mice (30 males and 30 females) were randomly selected and distributed into 12 groups,6 groups of males and 6 groups of females (five animals each).Both male and female groups were assigned to six treatments for three weeks as follows:1) the first group fed basal diet (control group); 2)the second group fed basal diet treated with low level of activated charcoal, 500mg/kg diet(AC_1 group); 3)the third group fed basal diet treated with high level of activated charcoal, 750mg/kg diet (AC_2 group); 4)the fourth group fed basal diet contaminated with aflatoxin AFB1, 40µg/kg diet (AF group); 5) the fifth group fed basal diet contaminated with aflatoxin AFB1 (40µg /kg diet) and treated with low level of activated charcoal, 500mg/kg diet (AF+AC_1 group); 6) the sixth group fed basal diet contaminated with aflatoxin AFB1(40µg/kg diet) and treated with high level of activated charcoal, 750 mg/kg diet (Af+AC_2 group).

At the end of the experimental period, mice were sacrificed by cervical dislocation for testing DNA damage, cytogenetic and reproductive studies. Two hours before sacrifice, mice were injected with 0.5mg of colchicines /kg b.w. for purpose of chromosome preparations.

DNA fragmentation

Liver samples were collected immediately after sacrificing the animals. The tissues were lysed in 0.5 ml lysis buffer containing 10 mM tris-HCL (PH.8), 1 mM EDTA, 0.2% triton X-100, centrifuged at 10000 r.p.m. (Eppendorf) for 20 minutes at 4°C. The pellets were resuspended in 0.5 ml of lysis buffer. To the pellets (P) and supernatants (S), 1.5

ml of 10% trichloroacetic acid (TCA) was added and incubated at 4°C for 10 minutes. The samples were centrifuged for 20 minutes at 10000 r.p.m (Eppendorf) at 4°C and the pellets were suspended in 750 µl of 5% TCA, followed by incubation at 100 °C for 20 minutes. Then to each sample 2 ml of DPA solution (200mg DPA in 10ml glacial acetic acid, 150 µl sulfuric acid and 60 µl acetaldehyde) was added and incubated at room temperature for 24 hour.[35] The proportion of fragmented DNA was calculated from absorbance reading at 600 nm using the formula:

$$\text{DNA fragmentation} = \frac{\text{OD of fragmented DNA(S)}}{\text{OD of fragmented DNA(S)} + \text{OD of intact DNA(P)}} \times 100$$

DNA extraction and Random Amplification of Polymorphic DNA (RAPD-PCR) analysis

Genomic DNA was isolated from liver tissue of male and female mice using phenol/chloroform method described by John et al.[36] To generate RAPD profiles from mice DNA, four commercially available decamer random primers (Operon, Almeda, CA, USA) were used including: **OPB10 (**5'CTGCTGGGAC3'**), OPA01 (**5'-CAGGCCCTTC-3'), **OPA05 (**5'-AGGGGTCTTG3'**), OPC02** (5'GTGAGGCGTC3'). The PCR protocol for RAPD analysis was followed as described by Williams et al.[29] Briefly, the amplification reactions were performed in a volume of (15µl) consisted of 1.5 µl (50 ng genomic DNA), 1.5 µl of 10X PCR reaction buffer, 1.5 µl DNTPs (200 µM), 1.5 µl primer (1pmol) (Operon, CA, USA), 1U Taq DNA polymerase. The final reaction mixture was placed in a DNA thermal cycler (Eppendorf). The PCR program included an initial denaturation step at 94°C for 4 min followed by 45 cycles with 94°C for 1 min for DNA denaturation, 1 min at 36°C, at 72°C for 2min and final extension at 72°C for 5 min were carried out. Approximately 3 µl of the amplified DNA product plus 2 µl of 1X loading dye were loaded on 2% agarose gel and then subjected to electrophoresis in 1X TBE buffer and stained with ethidium bromide (0.5µg/ml) for verification. A BIO-RAD XR$^+$ Molecular Imager apparatus was used to visualize the PCR products. The marker used is DNA ladder (3000-100pb) (Fermentas).

Micronucleus test

Bone marrow slides were prepared according to the method described by Krishna and Hayashi.[37] The bone marrow was washed with 1 ml of fetal calf serum and then smeared on clean slides. The slides were left to air dry and then fixed in methanol for 5 minutes followed by staining in May-Grunwald- Gemisa for 5 minutes then washed in distilled water and mounted. For each animal, 2000 polychromatic erythrocytes (PCEs) were examined for the presence of micronuclei.

Chromosome preparations

For chromosome analysis, the femurs were removed and the bone marrow cells were aspirated using saline solution in both treated and control animals. Metaphase spreads were prepared according to the methods of

Preston et al.[38] and Holden et al.[39] Fifty metaphase spreads per animal were analyzed, for scoring the different types of chromosome aberrations.

Sperm analysis

For sperm-shape analysis, the epididimus excised and minced in about 8 ml of physiological saline, dispersed and filtered to exclude large tissue fragments. Smears were prepared after staining the sperms with Eosin Y (aqueous), according to the methods of Wyrobek and Bruce[40] and Farag et al.[41] At least 3000 sperms per group were assessed for morphological abnormalities which were evaluated according to standard method of Narayana.[42] Epididymal sperm count was also determined by hemocytometer as described by Pant and Srivastava.[43]

Oocytes examination

Ovaries were collected from each female mouse and dissected to release the oocytes. The collected oocytes were numbered under a stereomicroscope. After that, the oocytes were fixed and stained with orcein stain to examine the meiotic progression under a phase contrast microscope.[44]

Statistical analysis

Statistical analysis was performed with SPSS software. Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's post hoc test for comparison between different treatment in the same sex. Moreover, T-test was used for comparison between male and female mice for inducing DNA fragmentation, micronuclei and chromosome aberrations in control and treated groups. Results were reported as mean ± S.E. and differences were considered as significant at p<0.05.

The polymorphism values were performed according to method of Taspinar et al.[45] as follows:

$$\text{Polymorphism value} = \frac{\text{Total No. of polymorphic bands of treated group}}{\text{Total No. of observed bands of control}} \times 100$$

Polymorphic bands = appearance of new bands (based on control bands) + disappearance of bands (based on control bands).

RESULTS

DNA fragmentation in male groups

The present results (Table 1) showed that the rates of DNA fragmentation were significantly (P<0.001) increased in male mice fed diet contaminated with aflatoxin (AF group) than those found in male mice fed basal diet alone (control group). Whereas, the addition of activated charcoal to diets contaminated with aflatoxin significantly decreased (P<0.05 or P<0.01) the rates of DNA fragmentation induced by aflatoxin diet alone. The supplementation of high level of charcoal to aflatoxin (AF+AC2 group) was more effective for decreasing the rate of DNA fragmentation than the supplementation of the low level of such adsorbent (AF+AC1 group) and there

were significant differences (P<0.05) between the two groups. On the other hand, the present results showed that the rates of DNA fragmentation were low in AC1 and AC2 groups and relatively similar with that found in the control group, statistical analysis indicated that there were no significant differences among the three groups.

Table 1: DNA fragmentation in male and female mice fed different diets

Sex	Treatment		Rates of DNA fragmentation
Male	Control		10.95 ± 0.26^{d}
	AC1		10.34 ± 0.25^{d}
	AC2		9.60 ± 0.24^{d}
	AF		52.88 ± 0.89^{a}
	AF+AC1		26.57 ± 0.49^{b}
	AF+AC2		21.70 ± 0.54^{c}
Female	Control		10.35 ± 0.22^{d}
	AC1		9.74 ± 0.35^{d}
	AC2		9.30 ± 0.20^{d}
	AF		47.71 ± 0.69^{a}
	AF+AC1		21.50 ± 0.45^{b}
	AF+AC2		17.50 ± 0.45^{c}
Comparison between males and females	Control	Male	10.95 ± 0.26
		Female	10.35 ± 0.22
	AC1	Male	10.34 ± 0.25
		Female	9.74 ± 0.35
	AC2	Male	9.60 ± 0.24
		Female	9.30 ± 0.20
	AF	Male	$52.88 \pm 0.89^{**}$
		Female	47.70 ± 0.69
	AF+AC1	Male	$26.57 \pm 0.49^{**}$
		Female	21.50 ± 0.45
	AF+AC2	Male	$21.70 \pm 0.54^{**}$
		Female	17.50 ± 0.45

AC1: basal diet treated with low level of activated charcoal

AC2: basal diet treated with high level of activated charcoal

AF: basal diet contaminated with aflatoxin AFB1

AF+AC1: basal diet contaminated with aflatoxin AFB1 and treated with low level of activated charcoal

AF+AC2: basal diet contaminated with aflatoxin AFB1 and treated with high level of activated charcoal; All data are expressed as mean±SE.

a, b, c, d, means with different letters are significantly different (P<0.05); ** Significant at P≤0.01

DNA fragmentation in female groups

DNA fragmentation results in female groups (Table 1) were similar with those recorded in male groups.

The comparison between male and female groups (Table 1) for inducing the DNA fragmentation, the results observed that the males had more rates of DNA fragmentation due to exposure to AF than females and there were significant differences (P<0.01) between the two sexes. However, the amelioration of the adverse

effect of aflatoxin by adding activated charcoal was clearer in females than those observed in males. Female groups, AF+AC1 or AF+AC2 had significant decreases (P<0.01) of DNA fragmentation as compared to male groups, AF+AC1 or AF+AC2, respectively.

Concerning the rates of DNA fragmentation of animals (males or females) fed basal diet treated only with activated charcoal, the results showed that the percentages of DNA fragmentation were low in AC1 or AC2 groups in the two sexes, and there were no significant differences between AC1, AC2 in male and female groups, respectively.

RAPD-PCR analysis

The obtained results from RAPD-PCR fingerprints generated by four primers, OPB10, OPA01, OPA05, and OPC02 for genomic DNA isolated from male and female mice are shown in Figure 1 and presented in Table 2. Each of the four primers displayed a strong amplification with distinct bands profile.

In male groups, the four primers amplified a total of 156 different bands. The molecular size of these bands ranged from 1749 to 218 bp. Of the 156 different bands, 82 were polymorphic. In all cases, polymorphisms in RAPD profiles were due to the loss (disappearance) and /or gain (new appearance) of the amplified bands in the treated samples in comparison to control RAPD profiles. The primer OPC02 revealed a highest level of polymorphism of 34 amplified bands of DNA.

The polymorphism value was found to be a higher level (87.5%) in aflatoxin group. However, the degree of polymorphism value was decreased in AF+AC1 (66.6%) and in AF+AC2 (62.5%) groups. The AF+AC2 treatment was more effective for decreasing the polymorphic level than the AF+AC1 treatment. On the other hand, the degree of polymorphism in mice fed basal diet treated

with AC2 was low (54.1%) than those found in AC1 group (70%).

In female groups, it can be seen that the four primers, OPB10, OPA01, OPA05 and OPC02 had generated a total of 129 different RAPD bands (Table 2). The molecular size of these bands ranged from 1583 to 300 bp. Of the 129 different bands, 45 were polymorphic. The primer OPB10 gave a highest level of polymorphism degree of 18 amplified bands of DNA.

AFB1 treatment caused a highest polymorphism value (52.1%). In contrast the treatments with AFB1 plus AC1 or with AFB1 plus AC2 showed a progressive reduction of polymorphism degree as compared to the treatment with aflatoxin alone (47.8% or 34.7% vs 52.1%). The treatment with AFB1 plus AC2 was more effective for reduction of polymorphism value than the treatment with AFB1 plus AC1. However, the degree of polymorphism in mice fed basal diet treated with AC2 was low (21.7%) than those observed (39.1%) in AC1 group.

From the present results as mentioned above, it was observed that the amplified RAPD bands obtained by four primers were similar in the two sexes of male (24 bands) and female (23 bands) control groups. However, the polymorphism values were higher in males than those found in females for all treatments.

Micronucleus assay in male groups

The present results (Table 3) observed that the frequencies of micro nucleated polychromatic erythrocyte (MNPCE) were significantly higher (P<0.001) in AF group than those found in the control group. Whereas, in AF +AC1 or AF+AC2 groups, the rates of MNPCE significantly decreased (P<0.05 or P<0.01) as compared to AF group alone. AF+AC2 group had the lowest percentage of micronuclei in comparison with AF+AC1 group, and there were significant differences (P<0.05) between the two groups.
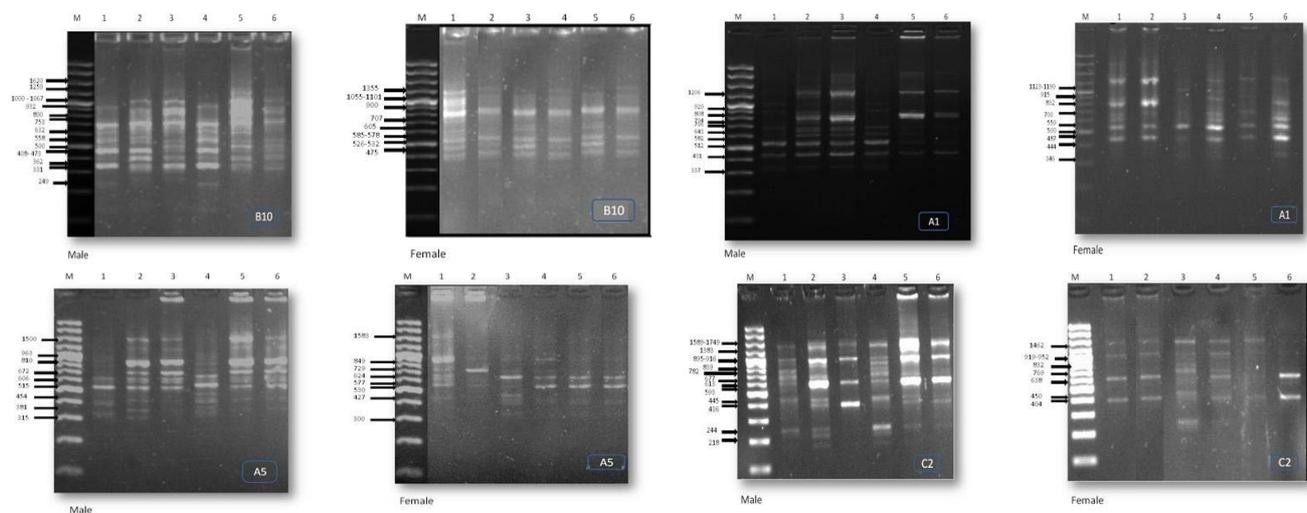

Figure 1: RAPD-PCR fingerprints generated by OPB10, OPA01, OPA05 and OPC02 for genomic DNA isolated from male & female mice. Lane M ladder DNA (3000-100pb). Lane 1(control), Lane 2(AC1), Lane 3(AC2), Lane 4(AF), Lane 5(AF+AC1), Lane 6 (AF+AC2).

Table 2: The total No. of bands in the control and polymorphic bands in mice fed different diets

Sex	Primer	Control	AC1		AC2		AF		AF+AC1		AF+AC2		Total no. of bands	Band size	Polymorphic bands
			a	b	a	b	a	b	a	b	a	b			
Male	B10	7	3	2	3	1	0	2	2	2	2	2	43	1620-249	19
	A1	6	1	1	2	0	4	2	1	0	1	2	40	1206-337	14
	A5	4	2	1	1	1	3	3	2	1	1	0	27	1500-315	15
	C2	7	3	4	3	2	4	3	4	4	5	2	46	1749-218	34
	Total	24	9	8	9	4	11	10	9	7	9	6	156		82(52.6%)
	a+b		17		13		21		16		15				
	polymorphism		70%		54.1%		87.5%		66.6%		62.5%				
Female	B10	6	1	2	2	2	1	3	2	2	2	1	34	1355-475	18
	A1	6	0	1	0	0	2	1	2	1	0	1	36	1190-346	8
	A5	7	1	1	0	1	0	3	0	2	0	2	34	1583-300	10
	C2	4	2	1	0	0	2	0	1	1	0	2	25	1462-404	9
	Total	23	4	5	2	3	5	7	5	6	2	6	129		45(34.9%)
	a+b		9		5		12		11		8				
	Polymorphism		39.1%		21.7%		52.1%		47.8%		34.7%				

a: appearance of new bands, b:disappearance of bands

Table 3: The frequencies of micronucleated polychromatic erythrocytes (MNPCE) in male and female mice fed different diets

Sex	Treatment	Mean values of MNPCE
Male	Control	3.20 ± 0.20^{d}
	AC1	3.20 ± 0.20^{d}
	AC2	3.20 ± 0.20^{d}
	AF	22.60 ± 0.81^{a}
	AF+AC1	17.40 ± 0.51^{b}
	AF+AC2	13.20 ± 0.37^{c}
Female	Control	2.80 ± 0.20^{d}
	AC1	2.80 ± 0.20^{d}
	AC2	2.20 ± 0.20^{d}
	AF	19.00 ± 0.32^{a}
	AF+AC1	14.20 ± 0.58^{b}
	AF+AC2	10.20 ± 0.37^{c}
Comparison between males and females	Control — Male	3.20±0.20
	Control — Female	2.80±0.20
	AC1 — Male	3.20±0.20
	AC1 — Female	2.80±0.20
	AC2 — Male	3.20±0.20*
	AC2 — Female	2.20±0.20
	AF — Male	22.60±0.81*
	AF — Female	19.00±0.32
	AF+AC1 — Male	17.40±0.51**
	AF+AC1 — Female	14.20±0.58
	AF+AC2 — Male	13.20±0.37**
	AF+AC2 — Female	10.20±0.37

There were 2000 polychromatic erythrocytes examined per animal; a, b, c, d, means with different letters are significantly different (P<0.05); *Significant at P≤0.05; **Significant at P≤0.01.

Concerning the groups of male mice that fed basal diet treated only with activated charcoal, the results showed that the rates of micronuclei were low in AC1 or AC2 groups and relatively similar with that found in the control groups, the statistical analysis showed that there were no significant differences among the three groups.

Micronucleus assay in female groups

The results of micronucleus assay in female groups were similar with those recorded in male groups.

The comparison between male and female groups (Table 3) for inducing the micronuclei, the results indicated that the males had more percentages of MNPCE due to feeding on aflatoxin diet than females and there were significant differences (P<0.05) between the two sexes. On the other hand, the improvement of the adverse effect of aflatoxin by treating with activated charcoal was more obvious in females than those observed in males. Female groups, AF+AC1 or AF+AC2 had significant decreases (P<0.01) of the rates of MNPCE in respect to male groups, AF+AC1 or AF+AC2, respectively.

Concerning the rates of MNPCE in groups of mice (males or females) fed basal diets treated only with activated charcoal, the results showed that the rates of MNPCE were low in each of AC1 male group and AC1 female group and there were no significant differences between the two groups. However, the percentages of MNPCE were slightly elevated in AC2 male group than those found in AC2 female group causing significant differences (P<0.05) between the two groups.

Chromosome examinations

Chromosome examination in male groups

The present results (Table 4) showed that the male mice fed diet contaminated with aflatoxin (AFB1) had

significantly elevation of the frequencies of structural and numerical chromosome aberrations than mice fed basal diet alone (control group). Whereas, the mice fed diet contaminated with aflatoxin and treated with low or with high level of activated charcoal (AF+AC1 or AF+AC2 groups) had significant decreases (P<0.05 or P<0.01) of the frequencies of chromosome aberrations as compared to mice fed diet contaminated with aflatoxin alone. The treatment with high level of activated charcoal was more effective for amelioration of the adverse effect of aflatoxin (AF+AC2 group) than the treatment with low level of activated charcoal (AF+AC1 group) and there were significant differences between the two groups for the frequencies of total structural (especially, deletions and fragments) and total numerical (aneuploidy and polyploidy) chromosome aberrations.

The results showed that the frequencies of chromosome aberrations in male mice fed basal diet treated with low (AC1 group) or with high (AC2 group) levels of charcoal were low and relatively similar with those observed in the control group, statistical analysis indicated that there were no significant differences between control and AC1 (or AC2) groups or between AC1 and AC2 groups.

Chromosome examination in female groups

Chromosome examination (Table 4) indicated that AF group had higher frequencies of structural and numerical chromosome aberrations than control group, and statistical analysis showed that there were significant differences between the two groups. In contrast, the treatment with activated charcoal diminished the adverse effect of aflatoxin and led to significant decreases (P<0.05 or P<0.01) of the frequencies of structural and numerical chromosome aberrations as compared to treatment with aflatoxin alone. The treatment with high level of activated charcoal to AF group (AF+AC2 group) was more effective for decreasing the chromosome aberrations than the treatment with low level of activated charcoal (AF+AC1 group) and there were significant differences between

the two groups for the frequencies of total structural (especially for fragments and centromeric attenuation (C.A.)) and total numerical chromosome aberrations.

The frequencies of chromosome aberrations in female mice fed basal diet treated with low (AC1 group) or with high (AC2 group) levels of activated charcoal were low and relatively similar with those found in the control group, statistical analysis showed that there were no significant differences between control and AC1 (or AC2) groups or between AC1 and AC2 groups.

In comparison between male and female groups, the results (Table 5) showed that the males had more response for AF-inducing chromosome aberrations than females. The male groups had the highest rate especially for structural and numerical chromosome aberrations as compared to female groups, and there were significant differences (P<0.01) between the two sexes.

The amelioration of adverse effect of aflatoxin by treating with activated charcoal was clearer in female groups than those of male groups. The female groups had the lowest rate of the chromosome aberrations in comparison with male groups and there were significant differences between the two sexes. The only exception to this, the frequencies of numerical aberrations in AF+AC2 male group were similar with those found in AF+AC2 female group and there were no significant differences between the two groups.

On the other hand, the frequencies of chromosome aberrations were low in each of AC1 male group and AC1 female group and there were no significant differences between the two groups. However, the frequencies of chromosome aberrations slightly raised in AC2 male group than those found in AC2 female group causing significant differences (especially for structural aberrations) between the two groups.

Table 4: The frequencies of chromosome aberrations in male and female mice fed different diets

Sex	Treatment	Structural aberrations							Numerical aberrations		
		Gaps	Breaks	Deletions	Fragments	C.A.	End.	Total	Aneuploidy	Polyploidy	Total
Male	Control	0.60 ± 0.25^c	0.40 ± 0.25^c	0.20 ± 0.20^c	0.00 ± 0.00^c	0.60 ± 0.25^d	0.20 ± 0.20^c	2.00 ± 0.32^d	0.60 ± 0.25^d	0.20 ± 0.20^c	0.80 ± 0.20^d
	AC1	0.80 ± 0.20^c	0.80 ± 0.20^c	0.40 ± 0.25^c	0.00 ± 0.00^c	0.60 ± 0.25^d	0.00 ± 0.00^c	2.60 ± 0.25^d	1.20 ± 0.20^d	0.20 ± 0.20^c	1.40 ± 0.25^d
	AC2	0.80 ± 0.20^c	0.80 ± 0.20^c	0.20 ± 0.20^c	0.00 ± 0.00^c	0.80 ± 0.20^d	0.00 ± 0.00^c	2.60 ± 0.40^d	0.80 ± 0.20^d	0.40 ± 0.25^c	1.20 ± 0.20^d
	AF	3.60 ± 0.40^a	3.40 ± 0.51^a	3.00 ± 0.32^a	2.40 ± 0.25^a	3.80 ± 0.20^a	1.80 ± 0.20^a	18.00 ± 0.77^a	4.20 ± 0.20^a	3.60 ± 0.51^a	7.80 ± 0.58^a
	AF+AC1	1.80 ± 0.20^b	1.80 ± 0.20^b	1.60 ± 0.25^b	1.00 ± 0.32^b	1.80 ± 0.20^b	0.80 ± 0.20^b	8.80 ± 0.20^b	2.60 ± 0.25^b	2.40 ± 0.40^b	5.00 ± 0.32^b
	AF+AC2	1.40 ± 0.25^{bc}	1.20 ± 0.20^{bc}	0.60 ± 0.25^c	0.20 ± 0.20^c	1.40 ± 0.25^{bc}	0.40 ± 0.25^{bc}	5.00 ± 0.45^c	2.00 ± 0.00^c	0.80 ± 0.20^c	2.80 ± 0.20^c
Female	Control	0.40 ± 0.25^c	0.40 ± 0.25^b	0.20 ± 0.20^b	0.00 ± 0.00^c	0.60 ± 0.25^c	0.00 ± 0.00^b	1.60 ± 0.25^d	0.60 ± 0.25^c	0.40 ± 0.25^c	1.00 ± 0.00^d
	AC1	0.40 ± 0.25^c	0.40 ± 0.25^b	0.40 ± 0.25^b	0.00 ± 0.00^c	0.60 ± 0.25^c	0.20 ± 0.20^b	2.00 ± 0.32^d	0.80 ± 0.20^c	0.20 ± 0.20^c	1.00 ± 0.32^d
	AC2	0.60 ± 0.25^c	0.40 ± 0.25^b	0.20 ± 0.20^b	0.00 ± 0.00^c	0.40 ± 0.25^c	0.00 ± 0.00^b	1.60 ± 0.25^d	0.40 ± 0.25^c	0.20 ± 0.20^c	0.80 ± 0.20^d
	AF	2.60 ± 0.25^a	2.00 ± 0.00^a	1.80 ± 0.20^a	1.80 ± 0.20^a	3.00 ± 0.32^a	2.00 ± 0.32^a	13.20 ± 0.37^a	3.60 ± 0.25^a	3.80 ± 0.20^a	7.40 ± 0.25^a
	AF+AC1	1.60 ± 0.25^b	1.00 ± 0.00^b	0.60 ± 0.25^b	0.60 ± 0.25^b	1.80 ± 0.20^b	0.60 ± 0.25^b	6.20 ± 0.37^b	2.20 ± 0.20^b	1.60 ± 0.25^b	3.80 ± 0.20^b
	AF+AC2	1.00 ± 0.00^{bc}	0.60 ± 0.25^b	0.60 ± 0.25^b	0.00 ± 0.00^c	0.80 ± 0.20^c	0.20 ± 0.20^b	3.20 ± 0.37^c	1.60 ± 0.25^b	1.20 ± 0.20^b	2.80 ± 0.37^c

C.A.: Centromeric attenuations; End.: Endomitosis; All data are expressed as mean±SE; a, b, c, d means with different letters are significantly different (P<0.05)

Table 5: Comparison between female and male mice for inducing chromosomal aberrations due to feeding on different diets

Treatment	Sex	Total structural aberrations	Total numerical aberrations
Control	Male	2.00±0.32	0.80±0.20
	Female	1.60±0.25	1.00±0.00
AC1	Male	2.60±0.25	1.40±0.25
	Female	2.00±0.32	1.00±0.32
AC2	Male	2.60±0.40*	1.20±0.20
	Female	1.60±0.25	0.80±0.20
AF	Male	18.00±0.78**	7.80±0.58
	Female	13.20±0.37	7.40±0.25
AF+AC1	Male	8.80±0.20**	5.00±0.32*
	Female	6.20±0.37	3.80±0.20
AF+AC2	Male	5.00±0.45**	2.80±0.20
	Female	3.20±0.37	2.80±0.37

* Significant at P≤0.05;** Significant at P≤0.01

Sperm examination

Sperm shape analysis

The present results (Table 6) showed that the sperm abnormalities (head and tail) were more frequent in AF group than those found in control group, and statistical analysis showed that there were significant differences (P<0.01 or P<0.001) for head abnormalities or (P<0.05) for tail abnormalities between the two groups. In contrast, the addition of activated charcoal to aflatoxin (AF+AC1 or AF+AC2) significantly decreased (P<0.05 or P<0.01) the sperm-shape abnormalities as compared to the treatment with aflatoxin alone. The supplementation with high level of charcoal (AF+AC2) was more effective for decreasing the sperm-shape abnormalities in respect to supplementation with low level of such adsorbent (AF+AC1) and there were significant differences for most sperm-shape abnormalities (except banana-like and tail abnormalities) between the two treatments.

Concerning the sperm abnormalities in male groups fed basal diet treated with activated charcoal, the results showed that the frequencies of sperm shape abnormalities were low in AC1 or in AC2 groups and they were relatively similar with those found in the control group, statistical analysis indicated that there were no significant differences among the three groups.

Sperm examination showed that sperm counts (Table 6) significantly (P<0.001) decreased in AF group in comparison with those found in the control group. Whereas, the sperm counts significantly ameliorated (P<0.05 or P<0.01) in AF+AC1 or in AF+AC2 groups as compared to those induced in AF group alone. The AF+AC2 group had the highest rate of sperm count in respect to AF+AC1 group and there were significant differences (P<0.05) between the two groups.

The sperm count was relatively similar in AC2 and control groups, while it was slightly decreased in AC1 group. Statistical analysis showed that there were no significant differences between control and activated charcoal groups (AC1 or AC2). However, the sperm count significantly increased (P<0.05) in AC2 group than those found in AC1 group.

Table 6: Sperm abnormalities in male mice fed different diets

Treatment	Head abnormalities						Tail abnormalities			T.S.A	Sperm count
	Amorphous	Without hock	Small	Big	Banana	Total	Divided	Coiled	Total		
Control	2.40±0.25[d]	2.00±0.32[d]	0.00±0.00[d]	0.00±0.00[c]	0.00±0.00[c]	4.40±0.40[d]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	4.40±0.40[d]	23.80±0.80[ab]
AC1	2.80±0.20[d]	2.60±0.40[d]	0.00±0.00[d]	0.00±0.00[c]	0.00±0.00[c]	5.40±0.40[d]	0.00±0.00[b]	0.60±0.40[b]	0.60±0.40[b]	6.00±0.63[d]	21.80±1.16[b]
AC2	2.20±0.20[d]	2.40±0.40[d]	0.00±0.00[d]	0.20±0.20[c]	0.00±0.00[c]	4.80±0.20[d]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	4.80±0.20[d]	24.80±0.58[a]
AF	34.00±0.70[a]	30.80±0.37[a]	10.60±0.40[a]	7.80±0.37[a]	4.40±0.25[a]	90.40±2.73[a]	1.20±0.37[a]	1.40±0.40[a]	2.60±0.25[a]	93.00±2.53[a]	8.60±0.51[e]
AF+AC1	20.60±0.25[b]	18.40±0.25[b]	7.80±0.37[b]	1.20±0.37[b]	0.60±0.25[b]	50.80±1.39[b]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	50.80±1.39[b]	15.80±0.37[d]
AF+AC2	14.00±0.32[c]	12.20±0.58[c]	4.20±0.74[c]	0.20±0.20[c]	0.40±0.25[bc]	31.00±0.63[c]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	31.00±0.63[c]	18.80±0.37[c]

T.S.A.: Total sperm abnormalities; All data are expressed as mean±SE; a, b, c, d, e, means with different letters are significantly different (P<0.05)

Oocytes competence *in vivo*

The collected number of oocytes

The numbers of recovered oocytes from female mice groups were summarized in Table 7. The present results showed that the collected number of oocytes were significantly (P<0.05) decreased in female group fed basal diet contaminated with aflatoxin (AF group) as compared to those found in the control. In contrast, the number of recovered oocytes of AF+AC1 or AF+AC2 groups significantly (P<0.05 or P<0.01) increased than those found in AF group alone. The AF+AC2 group had the higher number of the collected oocytes than AF+AC1 group and there was significant (P<0.05) difference between the two groups.

On the other hand, the number of collected oocytes of AC1 group was identical with that recovered in the control group, whereas, the AC2 group had significant (P<0.05) increase of the number of collected oocytes in comparison with either AC1 or control groups.

Oocytes maturation

The present results (Table 7) revealed that there were two types of the oocytes, immature oocytes in which the oocytes were at GVBD stage and mature oocytes that were at metaphase II stage (MII) or at telophase stage.

Statistical analysis showed that there were no significant differences between control and treated groups or among treated groups for the rate of immatured oocytes. Concerning the matured oocytes, the results indicated that the AF group had significant (P<0.05) reduction of the rate of matured oocytes as compared to the control group. In contrast, the rates of matured oocytes were significantly (P<0.05 or P<0.01) ameliorated in AF+AC1 and AF+AC2 groups in respect to AF group alone. The AF+AC2 group had the higher rate of matured oocytes as compared to AF+AC1 group, and there were significant (P<0.05) differences between the two groups.

The present results showed that the rate of matured oocytes in AC1 group was similar with that observed in the control and there were no significant differences between the two groups. Whereas, the AC2 group had significant (P<0.05) elevation rate of matured oocytes in comparison with AC1 or control groups.

Table 7: Nuclear stages in collected oocytes of female mice fed different diets.

Treatment	No. of oocytes	Nuclear stages (M± SEM)	
		Immature (GVBD)	Mature(MII + Telophase)
Control	20.00± 0.84[b]	8.00± 0.55[a]	12.00± 1.14[b]
AC1 diet	20.00± 0.70[b]	8.80± 1.11[a]	11.20± 1.07[b]
AC2 diet	25.60± 1.57[a]	6.60± 0.81[a]	19.00± 1.25[a]
AF diet	13.80± 1.36[c]	7.20± 0.80[a]	6.60± 0.82[c]
AF+AC1 diet	21.80± 1.02[b]	9.20± 1.07[a]	12.60± 1.86[b]
AF+AC2 diet	27.20± 1.24[a]	6.20± 1.11[a]	21.00± 0.45[a]

Matured oocytes: Oocytes at MII stage plus oocytes at telophase stage.
Immatured oocytes: Oocytes at GVBD stage; MII: MetaphaseII stage; All data are expressed as mean±SE; a,b,c means with different letters are Significantly different at P<0.05.

DISCUSSION

The mutagenic effect of aflatoxin

The present results showed that the mice fed diet contaminated with aflatoxin B1 had significant increase of DNA fragmentation as compared to control groups. These findings were similar with that reported by Eshak et al.[46] who found that the rates of DNA fragmentation were significantly increased in quail fed diet contaminated with aflatoxin B1 in respect to control group. In previous study, Gratz et al.[47] used DNA fragmentation as a marker of DNA damage in differentiated Caco-2 cells exposed to AFB1 following induction of CYP. AFB1 genotoxicity might be caused through the formation of AFB1-DNA adducts. AFs are metabolized by the mixed- function oxidase system to a number of hydroxylated metabolites and to aflatoxin[8,9] epoxide which binds to DNA, forming covalent adducts[6] and disturbs DNA replication causing DNA abnormalities.[7, 48,49] The adduct formation occurs preferably with guanine resulting in AFB1-N-7 guanine adduct responsible for mutagenesis in AFB1-treated cells.[50]

The present results revealed that, by using RAPD profile, the treatment with aflatoxin changed the polymorphism values at higher levels in male and female groups of mice as mentioned above (Table 2) in comparison with control profiles. AF toxicity is well known and has been widely reported in animals.[50-53] Concerning the molecular mechanism responsible for genotoxicity of aflatoxin , it has been suggested that AFs can induce a range of DNA damage such as single and double strand at different basic sites causing DNA adduct and resulting in high levels of genetic instability.[50-55] AFs genotoxicity may be mediated also indirectly; cell under oxidative stress display various dysfunctions due to lesions (such as bulky adducts or inhibition of DNA mismatch repair) caused by reactive oxygen species (ROS) to DNA.[52-56] Therefore, the observed changes in RAPD profiles in the present study may be due to DNA alterations induced by aflatoxin. Furthermore, in another studies, the changes in RAPD profiles by inducing high rate of polymorphism values of amplified bands of DNA were reported in some plants, these plants were exposed to another toxicants such as cadmium.[57-58]

The present results indicated that mice fed diet contaminated with aflatoxin B1 had significant elevation in the frequencies of micro nucleated polychromatic erythrocyte (MNPCE) than mice fed basal diet alone (control). These results were similar with those found by Eshak et al.[46] who reported that the frequencies of MNPCE were significantly increased in quail fed diet contaminated with AFB1 as compared to control. The interaction of AFB1 can be with the total genomic DNA. This can be result in small to large changes in genomic DNA including micronuclei and DNA fragmentation[50-55] as confirmed by our findings in the present study. Micronucleus assaying, as of chromosome aberrations, is a cytogenetic form that measures chromosomal damage, thus it is only effective when both DNA strands are broken.[59-60]

The present results showed clearly that AFs were genotoxic in bone marrow cells of mice. This genotoxicity of AFs revealed by inducing significant increases of structural and numerical chromosome aberrations in somatic cells of males and females as compared to normal control. These findings coincide with previous studies, Ezz El-Arab et al.[61], Darwish et al.[53] and Deabes et al.[34] who revealed that AFs (B1, B2, G1 and G2) administration significantly increased the frequencies of structural and numerical chromosome aberrations in bone marrow cells and in spermatocytes of male albino mice, in addition, mitotic and meiotic activities of somatic and germ cells were declined significantly. Moreover, Ghaly et al.[4] reported that AFB1 has induced different chromosome abnormalities in bone marrow cells and spermatocytes and shown to reduce the mitotic and meiotic activities of male albino mice. In rats, Ito et al.[62] and Abdel-Wahhab et al.[3] had previously reported that AFB1 has a potent activity to induce chromosome aberrations in bone marrow cells.

The mutagenicity of aflatoxin arising from the toxin molecules might form covalent N7 guanine-adducts which disturb DNA replication[63], resulting in anomalies in the chromosomes.[7] In addition, the AFB1-associated mutagenesis was suggested to represent a plausible cause for the higher chromosome instability observed in Chinese Hepatocellular Carcinomas, when compared with European primary liver carcinomas.[56] Several reports suggested that toxicity might ensue through the generation of intracellular reactive oxygen species (ROS), which may attack soluble cell compounds as well as membranes, eventually leading to the impairment of cell functionality and cytotoxicity.[51-56]

In the present results, the comparison between males and females, showed that males had more mutagenic response to aflatoxin than females, male groups had the highest rates of DNA fragmentation, polymorphism values of RAPD profile, MNPCE and chromosome aberrations. These findings are supported by report of Madle et al.[64] who used males and females of mice and rats with respect to their mutagenic response to the mycotoxin aflatoxin B1 (AFB1), the results showed that male animals exhibited higher frequencies of micronuclei than females, and a clear SCE- inducing effect was only detectable in males. These authors suggested that these differences might be due to metabolic differences between the two sexes, predominantly differences in glutathione conjugation of the reactive AFB1 epoxide and in the formation of the metabolic aflatoxicol. Moreover, Woo et al.[65] found that the incidence of hepatocellular carcinoma (HCC) due to exposure of AFB1 was much greater in adult male mice as infants than in females.

Regarding the reproductive toxicity, the present results clearly indicated that feeding on diet contaminated with aflatoxin caused adverse effects on male and female reproductive parameters in mice. AF caused a high significant elevation of morphological sperm abnormalities and a high significant reduction of sperm count in male mice. Also, in females AF treatment induced significant decreases in each of the number of collected oocytes and the rates of matured oocytes as compared to control. Concerning, sperm studies, various authors have reported similar kind of observations in different animals emphasizing AFs as reproductive toxicants[66], it induced a decrease in spermatogenic numbers in mice[67], decreased motility and longevity of breeding boar semen[68], as well as it caused disruption of spermatogenesis[55,69] and production of defective spermatozoa[70,71] when Swiss mice were treated with AFB1, the most potent and potentially lethal metabolite. In previous study, Fenske and Fink-Gremmels[72] reported that the toxin was found to impair spermatogenesis and cause accumulation of premeiotic germinal cells. Moreover, Darwish et al.[53] and Deabes et al.[34] revealed that AFs caused a high significant elevation of morphological sperm abnormalities and a high significant reduction of sperm count in male Albino mice.

The observed abnormal sperms in this study might be due to attack of generated ROS to polyunsaturated fatty acid residues of phospholipids of cell membrane occurring lipid peroxidation (LPO). Since the sperms have a high content of polyunsaturated fatty acids in the plasma membrane, they are highly sensitive to oxidative stress. Increased LPO and altered membrane can affect the sperm DNA leading sperm abnormalities.[73,74] DNA damage in gonadal cells of humans was due to excessive production of ROS by oxidative stress and led to damage in sperm morphology.[75,76]

Concerning the oocytes competence, to our knowledge there were very few data regarding reproductive effects of mycotoxin diets in females. Alam et al.[77] reported that oocyte quality is significantly reduced by feeding of mycotoxin to gilts. Where, the proportion of collected oocytes with compact cumuli reduced in gilts supplemented with mycotoxin contaminated feed in comparison to gilts fed on normal feed.

On the other hand, some investigations suggested that exposure to various environment factors could dramatically affect fertility. Studies of various contaminant-exposed wildlife populations indicated that multiple mechanisms contribute to changes in maturation of oocyte and fertilization.[78] Lipid peroxidation (LPO) compounds resulted from metabolites of toxicants including aflatoxins can influence biosynthetic pathways involved in both prostaglandin synthesis and steroidogenesis that have multiple roles in the regulation of reproductive function.[79] This LPO can modulate gene expression of ERK2 (termed extracellular signal regulated kinases that have been shown to have an important role during M-phase of cell division) during the oocyte maturation as a result of the influence of the steroidogenesis.[79-81] It is known that induction of the oocyte maturation is done through several compounds: luteinizing hormone (LH) induces the elevation time of the intracellular calcium required for resumption of meiosis. Calcium ions (ca^{2+}) act as a promoter to induce mRNA expression and the essential protein synthesis for the maturation processes. Therefore, oxidative compounds may modulate the gene expression through affecting the mechanism of action of LH and/or Ca^{2+}.[82-83]

The protective effect of activated charcoal

The present results showed that the activated charcoal treatment to contaminated diets with alfatoxin B1 significantly decreased the rates of DNA fragmentation, polymorphism proportions of RAPD profiles, the percentages of micronuclei MNPCE and frequencies of chromosome aberrations in male and female mice. Moreover, the addition of AC to AF diets significantly reduced the sperm abnormalities and significantly increased sperm count in males as well as it significantly enhanced both, the collected number of oocytes and the rate of oocytes maturation in females. In all cases the treatment with high level of AC was more effective for amelioration of the adverse effect of AF than the

treatment with low level of such adsorbent. Moreover, the ameliorative response for genetic parameters due to treatment with AC was clearer in females than those found in males and there were significant differences between the two sexes.

To our knowledge there is no published data showing the antagonism of AC against the adverse effect of AFs on genetic and reproductive constituents in mammals. The only exception to this, in another study, Azoz and Raafat[11] found in rats that the feeding on diet contaminated with lead and treated with AC significantly decreased the percentages of multinucleated polychromatic erythrocytes (MNPCE) than the feeding on diet contaminated with lead alone. However, the antagonism of AC against AFs and other toxicants is only studied on different animal species by using biochemical and protective performance parameters. In chickens, the addition of activated charcoal to AFB1-contaminated diets reduced the glutathione (GSH) content[84], decreased activity of microsomal enzymes and increased serum glutamic oxalacetic transaminase (SGOT) levels[85] in comparison with AFB1 diet alone. Also, in broilers the treatment with AC to AF diets improved feed consumption and body weight gain as well as reduced the mortality rate in broilers as compared to treatment with AF alone.[85,17] Moreover in another toxicants, Min and Young[86] found that AC decreased the increases of serum bilirubin and creatinine levels induced by lead in mice. Azoz and Raafat[11] reported in rats that the addition of AC to diets contaminated with lead significantly decreased the ALT and AST levels as well as significantly improved the red blood cells (RBCs) count and hemoglobin level (Hb) in rats in respect to feeding on diets contaminated with lead alone.

The protective effect of AC against toxicants toxicity may be due to the interaction between the adsorbent and toxicant. This adsorbent (AC) can remove toxicants from metabolically active cellular sites and reduce their intestinal absorption.[19,20] The amelioration of biochemical and productive performance parameters due to AC treatment are considered to be a marker of improvement of genetic and reproductive constituents. So, AC can be used as antioxidant and antidote in mammals.[11] In another study, the repeated administration of activated charcoal to patients with renal impairment could produce higher increases of digoxin clearance and reduce the toxic serum concentrations.[87]

In the present results, the comparison between males and females showed that females had more ameliorative response to AC treatment against the adverse effect of aflatoxin than males, where female groups had the lowest rates of DNA fragmentation, polymorphism values of RAPD profile, MNPCE and chromosome aberrations. These findings were similar with that reported by Plyem et al.[88] who studied the gender differences in the pharmacokinetics and Pharmacodynamics of drugs used in the practice of anesthesia, the results showed that females have 20-30% greater sensitivity to the muscle relaxant effects of vecuronim, pancuronim and rocuronium. Moreover, females are more sensitive than males to opioid receptor agonists as shown for morphin as well as for a number of Kappa (OP2) receptor agonists. The explanation to these gender differences was reported in some previous clinical studies. These studies indicated that many drugs that metabolized by CYP3A4, such as aflentanil and tirilazad , have demonstrated a 50-70% higher clearance in young females compared with young males.[89-91] This above discussion illustrate that gender should be taken into account as a factor that may be predictive for the dosage of several protectors. It may therefore be necessary to increase the dosage of the protectors to males compared with females in order to achieve similar recovery percentages against deleterious effects of toxicants.

In conclusion, the present study proved that AC has antagonistic or protective effect against AFB1. The degree of such protection of AC is probably related to its concentration ratio.

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